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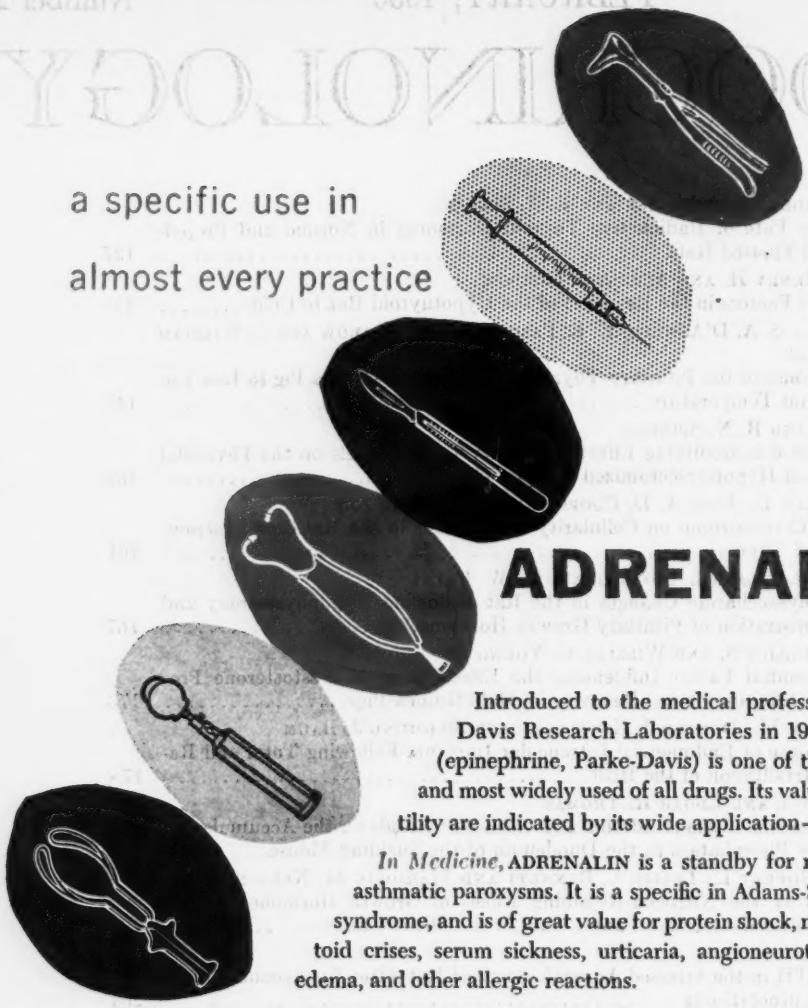

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METABOLIC FATE OF RADIOACTIVE THYROID HORMONES IN NORMAL AND PROPYL-THIOURACIL-TREATED RATS[1]

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INJECTION of I^{131}-labelled thyroxine into normal white mice (Gross and Leblond 1951a) was shown to result in the appearance, in all tissues studied, of at least four radioactive substances: thyroxine, iodide, and two unidentified compounds. One of these unidentified compounds was later shown to be 3:5:3′-triiodothyronine (Gross and Pitt-Rivers, 1952a, 1953a). Since then, clinical and experimental evidence has accumulated, suggesting that triiodothyronine is the form in which the thyroid hormone is active in other tissues (Gross and Pitt-Rivers, 1952b and 1953b; Gross, Pitt-Rivers and Trotter, 1952; Rawson, *et al.*, 1953; Lerman, 1953). However, no direct confirmation has been reported until recently (Kalant, Lee and Sellers, 1954; Flock and Bollman, 1954) of the occurrence of triiodothyronine in tissues or tissue fluids. The present communication is an amplification of the first of these recent abstracts.

The work reported here was undertaken as part of a study of the formation of tumors of the thyroid and pituitary glands of rats treated for prolonged periods with propylthiouracil (PTU) plus dried thyroid powder (Sellers, Hill and Lee, 1953). The distribution of metabolites of both thyroxine-I^{131} and triiodothyronine-I^{131} was studied in various tissues of normal rats and of those treated as mentioned above. The present paper describes the findings 24 hours after the injection of labelled hormones; these ob-

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[1] The experiments reported in this paper were supported in part by a grant from the National Cancer Institute of Canada.

[2] Senior Medical Research Fellow, the National Research Council of Canada.

servations may be of general interest in connection with the comparative actions of thyroxine and triiodothyronine. The results obtained at other time intervals, and their possible significance in the formation of tumors of the thyroid gland, will form the subject of a later communication.

EXPERIMENTAL

Twenty-four female rats of the Wistar strain, bred in the laboratory, were divided into equal groups and ketp in group cages. They were given food and water *ad libitum*.

One group, kept as normal controls, was fed the usual laboratory ration[3] in powdered form. The other group, referred to hereafter as the "treated group," was fed the powdered ration to which had been added 0.02% by weight of PTU and 0.02% of dried thyroid.[4] These doses were chosen because they had been found previously to maintain the metabolic rate of the rats within 10% of the normal average. The metabolic rate was determined at least twice for each animal during the course of the experiment.

The rats were maintained on this regimen for 10 months. Three animals from each group were then injected intraperitoneally with a quantity of I^{131}-labelled thyroxine[5] containing 100 μc. of I^{131} activity. Depending upon the specific activities of the various samples of hormone supplied, the total amount of thyroxine (or triiodothyronine[5] in the later experiments) in each dose was between 5 and 10 μg. In the case of thyroxine, this is less than one-fifth of the amount necessary to alter significantly the metabolic rate of normal rats of the size and strain used, when given as a single dose.

Twenty-four hours later, the animals were killed by ether, and were bled before removal of the pituitary, adrenals and thyroid. These organs were weighed using a fine torsion balance. Measured samples of the liver, gastrocnemius muscle, and blood plasma, were taken. The tissue samples, with the exception of the adrenals, were homogenized individually in a small Potter-Elvehjem homogenizer, and each was extracted three times with a total of 10 ml. of n-butanol. Following the butanol extraction, the solid residue from each sample was hydrolyzed in alkali, and the total hydrolysate and a measured aliquot of the butanol extract were used for measurements of I^{131} activity. From these results, the total activity of the original sample was calculated.

The remainder of each butanolic extract was taken to dryness *in vacuo*, at a temperature of 40°–60° C. Between 0.05 and 0.1 ml. of water was added to each, and the resultant solution was transferred by a capillary micro-pipette to the starting point of a two-dimensional paper chromatogram. Approximately 200 μg. amounts of the various carriers used—thyroxine, triiodothyronine, tyrosine and diiodotyrosine—were in turn applied to the same spot. Whatman #3 paper was used. The first solvent development was carried out in a 7:3 mixture of n-propanol:water. The second development was in a 75:25:5 mixture of butanone:water:acetic acid.

After development, each paper was clipped to a sheet of Ilford non-screen X-ray film, and left in the dark for three weeks to a month. The X-ray plates were then developed, and the carrier spots were located on the paper chromatograms by means of the diazotized sulphanilic acid spray described by Gross and Leblond (1951b).

The experiment was repeated at one-month intervals, using three rats from each group each time. In all, the procedure was carried out twice with radio-l-thyroxine and twice with radio-l-triiodothyronine.

---

[3] Fox breeder cubes, Master Feeds, Toronto Elevators, Ltd., containing 2.6 mg. iodine per 100 gm.

[4] Dried thyroid powder, containing 0.3% iodine. Parke, Davis and Co., Walkerville, Ontario.

[5] Obtained from Abbott Laboratories, Radioisotopes Division, Oak Ridge, Tennessee.

TABLE 1. I^{131} ACTIVITY OF VARIOUS TISSUES 24 HOURS AFTER INJECTION OF THYROXINE-I^{131}

Body wt. (gm.)	Thyroid gland				Liver			Muscle			Plasma
	Total wt. (mg.)	Gland wt. per 100 gm. body wt. (mg.)	Activity* of whole sample	Activity* per gm.	Sample wt. (mg.)	Activity* of whole sample	Activity* per gm.	Sample wt. (mg.)	Activity* of whole sample	Activity* per gm.	Activity* per ml.
					Control Group						
245	24.3	9.7	0.113	4.6	374	0.33	0.87	406	0.81	2.00	0.58
266	25.8	9.3	0.223	8.7	394	0.23	0.59	410	0.49	1.20	0.56
234	12.6	5.4	0.159	12.7	364	0.34	0.95	337	0.36	1.07	0.65
234	16.7	7.1	0.043	2.6	415	0.65	1.57	307	0.34	1.11	0.54
249	15.0	6.0	0.017	1.0	348	0.42	1.20	404	0.36	0.89	0.56
240	17.6	7.3	0.025	2.0	360	0.34	0.94	334	0.25	0.75	0.57
					Treated Group						
298	116.8	39.2	0.312	2.7	431	0.32	0.74	420	0.46	1.09	0.44
270	470.4	174.3	0.615	1.3	438	0.32	0.73	448	0.56	1.26	0.52
242	164.0	67.8	1.112	6.8	410	0.41	1.01	432	0.90	2.07	0.60
302	296.0	98.0	0.615	2.1	427	0.46	1.08	424	0.46	1.08	0.51
250	140.2	56.1	0.415	3.0	238	0.46	1.93	400	0.55	1.37	0.56
242	163.0	67.4	0.419	2.6	342	0.36	1.05	373	0.58	1.56	0.67

* All activities are expressed as per cent of the total administered dose.
Because of the small numbers in each group the results are considered to be semi-quantitative only.

RESULTS

The anatomical findings will not be described in detail, since they were in accord with those reported previously (Sellers, Hill and Lee, 1953).

In Table 1 are shown the results of I^{131} activity measurements in the various tissues examined, from both normal and treated rats injected with labelled l-thyroxine. Corresponding results for the animals injected with labelled l-triiodothyronine are shown in Table 2.

Thyroids. The I^{131} activity per gram of thyroid tissue (Table 1) was essentially the same in the two groups of animals, so that the total uptake in the enlarged thyroid glands of the treated rats was many times greater than in the normals. The difference was much more than could possibly be explained by difference in the amount of blood contained in the organ, since the activity of the plasma was low in both groups.

TABLE 2. I^{131} ACTIVITY OF VARIOUS TISSUES 24 HOURS AFTER INJECTION OF TRIIODOTHYRONINE-I^{131}

Body wt. (gm.)	Thyroid gland				Liver			Muscle			Plasma
	Total wt. (mg.)	Gland wt. per 100 gm. body wt. (mg.)	Activity* of whole sample	Activity* per gm.	Sample wt. (mg.)	Activity* of whole sample	Activity* per gm.	Sample wt. (mg.)	Activity* of whole sample	Activity* per gm.	Activity* per ml.
					Control Group						
254	17.2	6.8	0.129	7.5	337	0.116	0.345	378	0.043	0.114	0.08
225	17.4	7.7	0.165	9.5	298	0.121	0.406	323	0.020	0.070	0.17
237	18.0	7.6	0.171	9.5	294	0.125	0.426	370	0.035	0.094	0.11
248	18.2	7.3	0.121	6.7	391	0.167	0.427	406	0.026	0.064	0.08
260	20.4	7.8	0.011	0.5	351	0.131	0.374	376	0.046	0.122	0.12
243	18.2	7.5	0.014	0.8	362	0.141	0.390	370	0.028	0.076	0.14
					Treated Group						
250	2080.0	833.0	3.05	11.4	304	0.149	0.481	367	0.522	1.420	0.15
210	378.0	180.0	1.81	4.8	347	0.282	0.814	400	0.133	0.332	0.28
257	710.0	276.4	0.77	1.1	349	0.164	0.470	402	0.102	0.254	0.12
220	228.0	107.4	0.34	5.2	349	0.145	0.415	337	0.052	0.149	0.13
349	418.2	120.5	0.16	5.0	321	0.149	0.464	340	0.041	0.120	0.07
262	235.0	89.8	0.22	3.6	378	0.143	0.379	383	0.068	0.179	0.10

* All activities are expressed as per cent of the total administered dose.
Because of the small numbers in each group the results are considered to be semi-quantitative only.

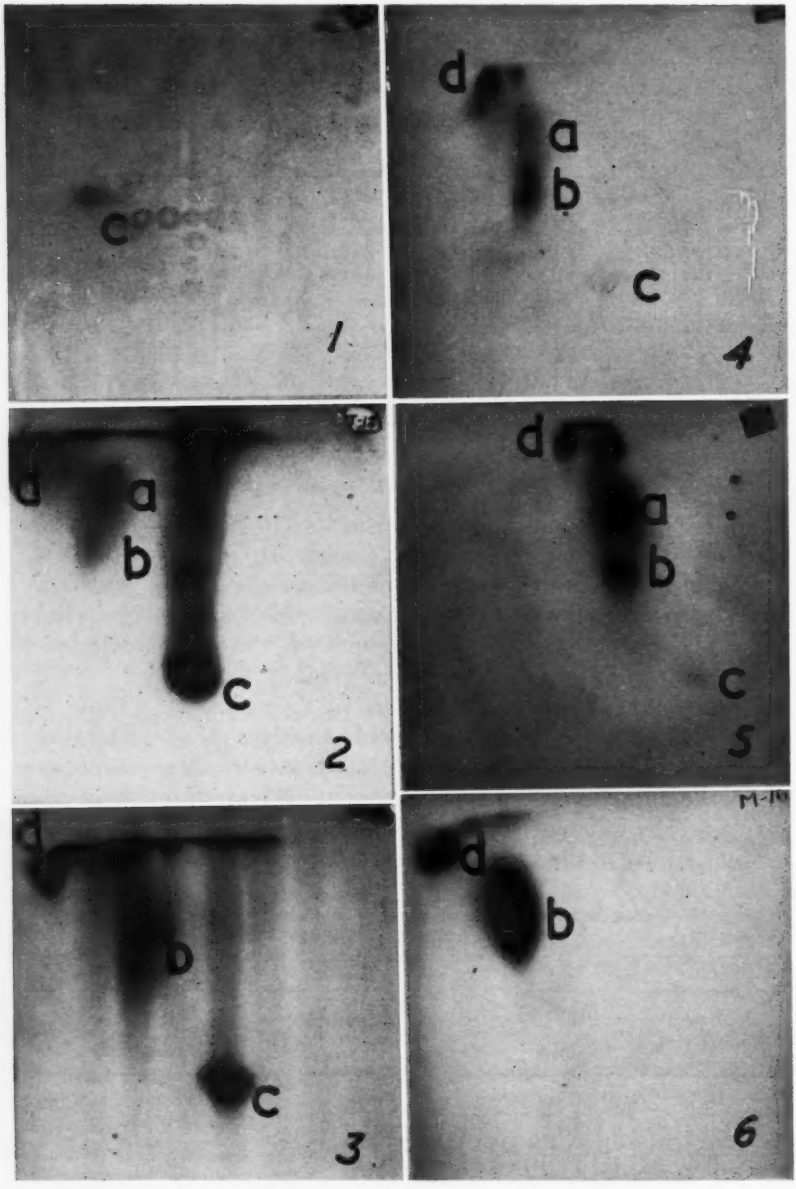

PLATE 1. RADIOAUTOGRAMS PREPARED FROM PAPER CHROMATOGRAMS OF TISSUE EXTRACTS.

FIG. 1. Thyroid of normal rat injected with thyroxine-I^{131}. Open circles are artefacts
FIG. 2. Thyroid of treated rat injected with thyroxine-I^{131}.
FIG. 3. Thyroid of treated rat injected with triiodothyronine-I^{131}.

Qualitatively, there was a further difference between the two groups of animals, with respect to the nature of the radioactive material found in the thyroid gland. Chromatograms and radioautographs of thyroid extracts from the normal animals, showed the radioactivity to be carried only by iodide and an unidentified spot near it (Fig. 1). In the radioautographs from the treated group (Fig. 2), however, the iodide spot was much stronger, and in addition all showed faint to moderately strong thyroxine spots. Three of the six showed spots in the location of triiodothyronine. One showed a very faint diiodotyrosine spot, and two showed an unidentified spot.

Similar findings with respect to both the radioactivity measurements, and the chromatographic distribution of radioactivity in the thyroid extracts, were obtained in the experiments with I^{131}-labelled triiodothyronine. The greatly enlarged thyroids of the treated animals again showed similar activities per gram of tissue to those of the normals, but a much higher total radioactivity. Qualitatively, the normals showed all the radioactivity in the location of iodide on the chromatograms, whereas the treated group showed activity in both the iodide and triiodothyronine spots (Fig. 3).

Pituitaries. Total radioactivity measurements in the pituitaries failed to show any counts appreciably above background, in any of the groups. One attempt at chromatography and radioautography of the extracts gave completely blank plates.

Liver. As the weights of the whole livers were not obtained, the total uptakes could not be calculated. Subject to this limitation, no difference, either quantitative or qualitative, was observed between the uptakes of radioactive material by the livers of the normal and treated rats, following the injection of either thyroxine-I^{131} or triiodothyronine-I^{131}. The activities per gram of tissue, in both groups in the triiodothyronine experiments, were only about one-third as great as those found in the labelled thyroxine experiments.

This difference was paralleled by that seen in the radioautographs of the liver extract chromatograms. In all the radiothyroxine series, both normal and treated, there were at least four radioactive spots apparent, corrresponding to the administered thyroxine, triiodothyronine, iodide, and one or more unidentified substances.

←

FIG. 4. Muscle of normal rat injected with thyroxine-I^{131}.
FIG. 5. Muscle of treated rat injected with thyroxine-I^{131}.
FIG. 6. Muscle of treated rat injected with triiodothyronine-I^{131}.

Labelled spots correspond to: a) thyroxine, b) triiodothyronine, c) iodide, d) lipid material.

Unlabelled spots were unidentified.

In the triiodothyronine series, only two spots were seen on each radioautograph; these occupied the positions taken up by triiodothyronine and by iodide.

Skeletal muscle. As in the case of the liver samples, only the activity per gram of tissue is shown in Tables 1 and 2.

In the thyroxine-I^{131} series, the normal and treated animals showed the same range of activities. This was paralleled by a similar distribution of radioactive spots on the muscle extract chromatograms. At least four spots appeared on each radioautograph: moderate to strong spots of thyroxine and triiodothyronine, and much fainter spots corresponding to iodide and possibly to Gross and Leblond's "unknown 4." The triiodothyronine spot was clearly the strongest in all, but more so in the treated group (Fig. 5) than in the normals, (Fig. 4).

In the triiodothyronine-I^{131} series, the activities per gram of tissue, in both groups, were much lower than the corresponding values in the thyroxine-I^{131} series. Moreover, the muscle of the treated rats showed a considerably higher activity than that of the normal rats. A similar relationship obtained in the radioautographs. The normals showed only faint to moderately intense triiodothyronine spots, while the treated group (Fig. 6) showed heavier triiodothyronine spots, and faint iodide and other spots similar to those already described for other tissues.

Lipid spots. In the chromatograms of all tissues examined, from all the animals, an irregular yellow zone was seen along the butanone-water-acetic acid solvent front, especially marked at the intersection of the two solvent fronts. This zone contained a water-repellent material which could be easily eluted with a 3:1 mixture of ethanol:acetone, suggesting that it was of lipid nature. The eluted material give a positive Liebermann-Burchard reaction, indicating the probable presence of cholesterol. Tests for free iodine and for iodide were negative in this zone.

DISCUSSION

The findings reported above are in essential agreement with those of Gross and Leblond (1951a), with respect to the metabolites of thyroxine in skeletal muscle and liver. As qualitatively similar findings have thus been reported for the mouse, the rat and the dog (Flock and Bollman, 1954), there is some basis for considering the appearance of triiodothyronine in the tissues, following the administration of thyroxine, to be a general phenomenon.

In the present study, the administration of labelled triiodothyronine resulted in a distribution of radioactive metabolites in peripheral tissues, which was qualitatively similar to that found after giving labelled thyroxine, with the exception that no radioactive thyroxine was found after administration of triiodothyronine. There is no evidence that deiodination of thyroxine actually occurred in the muscle cells. However, the intensity

of the triiodothyronine spots found after administration of both labelled hormones would tend to support the idea that triiodothyronine is the peripherally utilized form of thyroid hormone, whether or not it is formed at the site of its action.

Although the same dose of radioactivity was administered in all cases, whether as triiodothyronine or as thyroxine, considerable differences were encountered in the activities of comparable tissues, 24 hours after the injection of the two substances. These differences may reflect corresponding differences in the rate of uptake and utilization of the two substances by the tissues examined. It is possible that triiodothyronine passes its peak concentration in peripheral tissues considerably earlier than does thyroxine. Studies are now in progress to clarify this point by examination of radioactivity of these various tissues at a series of short time intervals following the injection of thyroxine-I^{131} and triiodothyronine-I^{131}.

The only striking differences encountered between the normal and treated rats were in respect of the findings in the thyroid gland. The peripheral tissues showed no apparent differences in either total radioactivity or the qualitative distribution of radioactive constituents. In the thyroid glands, however, the treated group showed a much higher total radioactivity than the normals, but the activities per gram of tissue did not differ significantly. Qualitatively, the radioactivity in the extracts of normal glands was all borne by inorganic iodide, while in the treated group the extracts contained radioactive thyroxine and triiodothyronine. These findings could permit of at least two possible interpretations: (1) the glands of the treated animals took up intact exogenous thyroid hormones, or (2) the inhibition of the glands by PTU was incomplete, and some synthesis of labelled hormones took place from radioactive iodide taken up by the gland, after breakdown of the administered hormone elsewhere in the body. The latter possibility seems less likely, as no labelled thyroxine or triiodothyronine was found in the normal glands at this time interval, even though labelled iodide was present. However, no definite answer is given by the present work, and the possible significance of the findings with respect to tumor formation in the treated group must await clarification of the point.

An interesting finding in these studies has been the presence in the chromatograms of all tissues studied, of a zone of radioactive lipid material, possibly of sterol nature. This may be an artefact of extraction, resulting from iodination of unsaturated bonds, but there is a possibility that it represents an intracellular metabolic conversion product of the thyroid hormone, and such a possibility merits further study.

SUMMARY

Wistar rats treated with propylthiouracil plus dried thyroid powder for 10 months, and normal control animals, were injected with thyroxine or triiodothyronine labelled with I^{131}. After 24 hours, their thyroid, liver, skeletal

muscle and pituitary tissues were extracted, and the amounts and identities of free radioactive substances were determined.

In all groups, skeletal muscle and liver were found to contain appreciable amounts of the respective labelled thyroid hormones administered. Following the injection of labelled thyroxine, triiodothyronine was one of the principal radioactive substances identified in these tissues. Similar findings were obtained in the thyroid glands of the rats treated with propylthiouracil and thyroid powder, whereas the thyroid glands of the normal rats showed radioactivity almost exclusively in the inorganic iodide fraction. No appreciable radioactivity could be detected in the pituitary glands.

Acknowledgment

The authors wish to acknowledge generous donations of l-thyroxine from the Glaxo Laboratories Ltd. of Great Britain through Glaxo (Canada) Limited, and of propylthiouracil from the Lederle Laboratories Division of the American Cyanamid Company.

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ENDOCRINE FACTORS IN THE RESPONSE OF THE HYPOTHYROID RAT TO COLD

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FOLLOWING either thyroidectomy or adrenalectomy the rat fails to survive exposure to conditions of low environmental temperature that are tolerated indefinitely by the intact animal.

Leblond and Gross (1943) noted that thyroidectomy reduced, but did not prevent, the increase in adrenal weight exhibited by normal rats exposed to cold. Ershoff (1948) described the failure of adrenal cortical extract to prolong the survival of thiouracil-treated rats in cold, in which experiments thyroxin, in microgram quantities, allowed 100% survival. These findings suggest that the decrease in adrenal cortical activity (Leblond and Hoff, 1944; Baumann and Marine, 1945; Freedman and Gordon, 1950) plays a secondary role to the thyroid in the response of the hypothyroid rat to cold.

Zarrow and Money (1949) found a direct correlation between adrenal size and survival time in thiouracil-treated rats. Blockage of the formation of 11-oxycorticosteroids, either through a lack of ACTH or because of an inability of the adrenal cortex to respond to ACTH was suggested as the explanation for the greater mortality of the thiouracil-treated group. Gabrilove and Soffer (1950) demonstrated that the administration of epinephrine or of ACTH to propylthiouracil-treated rats resulted in depletion of adrenal ascorbic acid equivalent to that found in the normal controls; following continued ACTH treatment adrenal hypertrophy of the same magnitude as in the normal rat was also obtained. The latter result was noted also by Zarrow and Zarrow (1951) who suggested that the failure to the hypothyroid rat to survive cold exposure, and adrenocortical involution at normal temperature, followed from the limited number and types of cells in the anterior pituitary which was elaborating thyrotrophin at the expense of ACTH.

The experiments reported herein were undertaken to determine whether

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[1] Taken from a thesis presented by Henry H. Freedman to the Faculty of the Graduate School of Arts and Science, New York University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology.

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the pituitary-adrenocortical axis of the hypothyroid rat can respond to physiological stress (*e.g.*, cold), and, if so, whether the response is abnormal. Further, the effects of various hormones were determined upon survival time of the thiouracil-treated rat.

METHODS

Young male rats of a modified Long-Evans strain were used throughout. Prior to exposure to cold the animals were kept at room temperature, and received a powdered complete ration and water *ad libitum*. Those to be rendered hypothyroid were given the same diet with 0.2% thiouracil thoroughly mixed into their powdered meal. For cold exposure the animals, in pairs in metal cages, were placed in a refrigerator (temperature: 5° ± 1.5° C.), and were observed at frequent intervals. Except where otherwise noted, food and water were provided during the periods of subjection to cold.

Since the rats on the goitrogen do not grow as rapidly as the normal animals (Gordon, Goldsmith and Charipper, 1946), and since survival time in cold is related to body weight (Zarrow and Money, 1949), comparisons were made between control and experimental animals of the same final body weight.

In the first set of experiments 86 animals were employed. Organ weights and adrenal ascorbic acid and ester cholesterol concentrations were determined in normal rats at 25° ± 2° C. and when exposed to 5° C. for 1, 3, 18, and 24 hours. Other rats treated with thiouracil for 17 days were examined at room temperature and after 1 and 24 hours in the cold, and another set kept on the goitrogen for 12 weeks were followed as were the normal groups.

In the second experiment 74 animals were clipped of body hair (Sellers, You and Thomas, 1951) one hour before exposure. The hypothyroid rats had been on the thiouracil-supplemented diet for 28 days prior to the experiment. Survival time was determined for normal and treated animals, and, in addition, determinations were made on hypothyroid groups receiving the following supplementary treatments: 1) Lipo-Adrenal Cortex (Upjohn), 0.5 ml./100 gm. body weight daily, administered subcutaneously for 3 days starting 2 days before exposure; 2) d,l-thyroxin, 10 μg./100 gm. body weight daily, subcutaneously for three days; 3) epinephrine, 0.5 ml. 1:1000, subcutaneously just before exposure; 4) ACTH aqueous (E. R. Squibb & Sons), 3 I.U. twice daily, subcutaneously for 6 days before exposure; 5) ACTH in gelatin (Princeton Laboratories, Inc.) 0.5 ml. (10 I.U.) daily, subcutaneously for 6 days; 6) ascorbic acid, 50 mg. daily, orally, for 6 days before exposure. A group of hypothyroid rats receiving thyroxin was sacrificed after 4 hours in the cold, the approximate survival time of the other groups. In this experiment, most determinations were obtained from rats in a terminal state.

In a last set of experiments 24 animals were employed; half were maintained on a normal diet and half received the thiouracil feed for 28 days. Half of each group remained at room temperature as controls, and the others were kept at 5° C. for 96 hours.

All animals were sacrificed by exsanguination and the organs trimmed of adhering tissue, and weighed on a torsion balance to the nearest 0.1 mg. Ascorbic acid was determined by the method of Roe and Kuether (1943) with the dinitrophenylhydrazine reagent prepared according to Lowrey, Lopez and Bessey (1945). For ester cholesterol a modification of the method of Schoenheimer and Sperry (1934) was employed.

RESULTS

Adrenal response to cold

The data are given in Table 1. In the normal rat, it may be seen that the adrenal ascorbic acid concentration decreased after 1 hour exposure at

TABLE 1. EFFECTS OF EXPOSURE TO 5° C. FOR 1, 3, 18 AND 24 HOURS ON ADRENAL WEIGHTS AND ADRENAL ASCORBIC ACID AND ESTER CHOLESTEROL IN THE NORMAL AND IN THE HYPOTHYROID RAT

Group	Hours in cold	Body[1] weight gm.	Thyroid weight mg.	Adrenal weight mg.	Ascorbic acid, μg./mg. adrenal	Ester cholesterol, μg./mg. adrenal
Normal						
(15)[2]	0	154	16.1 ± 0.5	23.1 ± 0.9	4.79 ± 0.15	15.4 ± 1.4
(10)	1	153	15.5 ± 0.9	21.4 ± 0.8	3.25 ± 0.17	
(3)	3	161	14.9 ± 1.4	23.9 ± 1.9	4.86 ± 0.27	7.4 ± 0.9
(3)	18	159	16.2 ± 2.6	26.4 ± 2.2	5.56 ± 0.37	5.4 ± 1.9
(8)	24	169	16.6 ± 1.0	24.8 ± 1.0	4.62 ± 0.12	
Hypothyroid, 0.2% Thiouracil, 17 Days						
(6)	0	143	40.7 ± 2.8	18.5 ± 1.3	4.82 ± 0.10	
(6)	1	142	38.1 ± 2.0	17.7 ± 0.7	2.88 ± 0.20	
(6)	24	164	43.2 ± 3.8	21.3 ± 1.4	5.06 ± 0.11	
Hypothyroid, 0.2% Thiouracil, 12 Weeks						
(9)	0	159	74.7 ± 6.8	16.9 ± 0.5	5.02 ± 0.18	13.1 ± 1.0
(6)	1	166	59.9 ± 9.2	17.8 ± 0.8	2.43 ± 0.18	
(3)	3	161	79.6 ± 12.3	16.4 ± 1.7	2.79 ± 0.20	2.9 ± 1.6
(3)	18	163	80.1 ± 16.4	18.9 ± 2.0	3.82 ± 0.23	2.3 ± 1.1
(8)[3]	24	170	75.0 ± 6.1	20.7 ± 0.9	2.95 ± 0.20[4]	2.8 ± 1.2[4]

Values are Means ± Standard Errors.
[1] At sacrifice.
[2] Numbers of rats shown in parentheses.
[3] 6 of 8 animals dead at 24 hours.
[4] Values for 2 surviving animals.

5° C. and returned to, or above, normal values after 18 and 24 hours in the cold. For the animals on 0.2% thiouracil for the brief period of 17 days there was no significant difference in the vitamin C concentration in the adrenals before exposure to cold; moreover the depletion after 1 hour exposure was fully as great as in the normal rats. At 24 hours, with all animals surviving, the ascorbic acid level had returned to normal.

Following 12 weeks on the goitrogenic diet, the pre-cold ascorbic acid values were normal and the fall in concentration after 1 hour in the cold was greater than in the other two groups. Seventy-five per cent of the animals subjected to this treatment failed to survive 24 hours at 5° C., and in the survivors there was no return of adrenal ascorbic acid to normal; at 3 and at 18 hours the levels were still low as compared to the quick recovery shown by the normal rats. This failure to recover adrenal ascorbic acid is typical of severe stress ending in death (Sayers and Sayers, 1948).

The changes in ester cholesterol paralleled those described above for vitamin C, except for the well known slower time course. In the normal groups this adrenal constituent decreased by 65% at 18 hours exposure and by 83%, at this time, in the animals on the 12 week thiouracil-supplemented diet.

Comparison of the 24 hour groups with their respective controls, for the three series, reveals no significant change in adrenal weights when these are related to unit body weight.

The progressive atrophy of the adrenals (Table 1), the thyroid enlargement, and the increase in pituitary weight (approximately 30%) induced

by thiouracil are well known and have been confirmed in the present work. There was a marked reduction in the weight of the thymus, from 143 mg./100 gm. body weight to 72 mg./100 gm., and of the spleen, from 580 mg./100 gm. to 246 gm./100 gm., following 12 weeks on thiouracil.

Hormonal effects upon survival time

As seen in Table 2, the animals treated with 0.2% thiouracil for 28 days had pre-cold adrenal ascorbic acid and ester cholesterol concentrations not different from the untreated controls. The clipped hypothyroid rats sur-

TABLE 2. SURVIVAL AT 5° C., ORGAN WEIGHTS AND ADRENAL ASCORBIC ACID AND ESTER CHOLESTEROL IN BOTH THE NORMAL AND HYPOTHYROID RAT CLIPPED OF BODY HAIR, INCLUDING THE EFFECTS OF SUPPLEMENTARY TREATMENT

Group	Hours in cold	Body[1] weight gm.	Thyroid weight mg.	Adrenal weight mg.	Ascorbic acid, μg./mg. adrenal	Ester cholesterol, μg./mg. adrenal	Thymus weight mg.
Normal							
(11)[2]	0	174	15.9±1.0	26.4±0.8	4.80±0.14	17.9±0.6	231.1±6.1
(5)	4[3]	142	16.4±0.7	23.8±0.9	4.10±0.21	12.3±0.9	205.0±9.4
(5)	48[4]	159	16.5±1.2	28.8±1.1	4.71±0.19	5.0±1.1	157.5±8.7
Hypothyroid, 0.2% Thiouracil, 28 Days							
(9)	0	163	56.3±1.2	19.0±0.9	4.63±0.12	17.6±1.3	136.2±4.3
(9)	3–5[5]	162	55.9±5.4	18.8±0.8	2.88±0.07	9.8±1.3	139.1±5.2
Hypothyroid, plus Adrenal Cortical Extract							
(6)	3–5[5]	168	50.1±2.9	19.3±0.9	2.87±0.12	7.9±1.3	90.0±2.3
Hypothyroid, plus ACTH (aqueous)							
(4)	3–5[5]	174	56.4±3.7	20.0±1.1	3.15±0.21	5.0±0.9	107.5±5.9
Hypothyroid, plus ACTH (gelatin)							
(4)	3–5[5]	183	54.3±5.1	30.5±1.2	2.98±0.11	9.5±1.4	82.7±7.5
Hypothyroid, plus Ascorbic Acid							
(6)	3–5[5]	182	51.6±3.3	20.9±1.3	2.94±0.09	11.9±1.4	91.4±6.4
Hypothyroid, plus Epinephrine							
(5)	3–5[5]	163	59.9±1.3	20.6±1.8	2.83±0.10	6.7±0.4	147.4±9.0
Hypothyroid, plus Thyroxin							
(6)	4[3]	171	53.7±3.1	28.6±1.0	4.09±0.11	13.6±0.8	128.4±5.5
(4)	48[4]	155	60.2±3.7	33.1±1.3	4.49±0.15	4.0±0.6	118.0±8.6

Values are Means±Standard Errors.
[1] Body weight at sacrifice.
[2] Numbers of rats shown in parentheses.
[3] Time of sacrifice.
[4] Sacrificed at from 24 to 48 hours.
[5] Time of survival.

vived for 3 to 5 hours in the cold. During the first 2 to 3 hours their behavior could not be distinguished from that of normal rats but there followed a rapid deterioration terminating in coma and death. When adrenal analysis was performed upon animals in a terminal state, ascorbic acid had fallen by 38% and ester cholesterol by 44%.

The supplementary treatment of the clipped hypothyroid animals with adrenal cortical extract, epinephrine, ACTH, or with ascorbic acid prior to cold exposure did not increase survival time. The depletion of adrenal ascorbic acid occurred to the same degree in all of these groups. The drop in ester cholesterol concentration can be seen to be more variable, but without significant differences.

Pre-treatment of these animals with ACTH in gelatin caused a significant increase in adrenal weight (30.5 *vs* 19.0 mg.). Thymus weight was reduced by the cortical extract by 34% and by the ACTH in gelatin by 40%, both values compared to the already involuted thymus of the thiouracil-treated controls.

In contrast to the above, the clipped normal rat survived the low temperature for the maximum of 48 hours allowed. For clipped animals sacrificed at from 24 to 48 hours exposure there was an increaase in adrenal weight during the cold stress (18.1 *vs* 15.2 mg./100 gm. body weight). The thymus was reduced from 231 to 157 mg. Adrenal ascorbic acid had returned to normal but ester cholesterol was severely depleted (by 72%).

The group of clipped normal rats sacrificed at 4 hours for comparison of adrenal chemistry with the groups failing to survive longer than this showed only a slight decrease in ascorbic acid (14%) and in cholesterol-ester (31%).

The only treatment that allowed normal survival in the thiouracil-treated rats was direct replacement therapy with thyroxin. For both sets so treated, sacrificed at between 24 and 48 hours and at 4 hours of cold exposure, the adrenal changes in vitamin C and ester cholesterol closely duplicated those obtained in the normal rats.

It can be noted that pre-treatment with ascorbic acid did not prevent the depletion of this substance from the adrenals of the hypothyroid rat in the cold, although the effect upon adrenal cholesterol was a sparing one. Similarily, pre-treatment with adrenal cortical extract did not alter the extent of the depletion of the measured constituents in these animals at low temperature.

Continuous 96 hour exposure

In this experiment (Table 3) it is seen that significant adrenal hypertrophy occurred in both hypothyroid and normal rats exposed continuously to low environmental temperature for 96 hours. Although 2 of the 6 thiouracil-treated rats failed to survive the 96 hours, the adrenal weight for the group rose from 22.5 to 29.4 mg., an increase of 31% ($t=5$). The comparative figures for the untreated group were 27.9 to 35.2 mg. an increase of 26% ($t=4$).

The hypothyroid rats had significantly lower pre-cold levels of adrenal ester cholesterol, and, at the end of the cold stress, there was a severe depletion that was not found in the normal group. The adrenal ascorbic acid changes after cold were not as markedly different in the normal and hypothyroid groups.

Thymic involution, here again, was greater in the untreated rats but in view of the pre-experimental thymic reduction caused by thiouracil, the cold involution from 101 to 75 mg. ($t>2.5$) is striking.

TABLE 3. EFFECTS OF EXPOSURE TO 5° C. FOR 96 HOURS ON ADRENAL AND THYMUS WEIGHT AND ADRENAL ASCORBIC ACID AND ESTER CHOLESTEROL IN THE NORMAL AND IN THE HYPOTHYROID RAT

Group	Adrenal weight mg.	Ascorbic acid, μg./mg. adrenal	Ester cholesterol, μg./mg. adrenal	Thymus weight mg.
1. Normal, not exposed to cold	27.9 ± 1.5	4.73 ± 0.16	22.7 ± 1.4	250.2 ± 13.8
2. Normal, sacrificed at 96 hours	35.2 ± 0.9	4.59 ± 0.22	21.4 ± 0.7	135.1 ± 9.2
3. Hypothyroid, not exposed to cold	22.5 ± 1.0	4.54 ± 0.13	13.3 ± 1.0	101.5 ± 4.7
4. Hypothyroid, sacrificed at 96 hours	29.4 ± 1.1	3.56 ± 0.40	2.8 ± 0.3	75.0 ± 7.9

Values are Means ± Standard Errors.
6 animals in each group.
Group 1. body weight = 198 gm.; thyroid = 16.1 ± 0.7 mg.
Group 2. body weight = 199 gm. at start, loss = 4 gm.; thyroid = 17.5 ± 0.6 mg.
Group 3. received 0.2% thiouracil for 32 days; body weight = 196 gm.; thyroid = 45.7 ± 1.2 mg.
Group 4. received 0.2% thiouracil for 28 days prior to start of cold exposure and continued during experiment; body weight = 200 gm. at start, loss = 26 gm.; thyroid = 43.0 ± 1.6 mg.; 2 of 6 animals dead at 96 hours.

DISCUSSION

From the results obtained in the first experiment there appears to be no lack in the thiouracil-treated animals of an acute pituitary-adrenocortical response, as measured by changes in adrenal ascorbic acid and ester cholesterol concentrations. If, as suggested by Zarrow and Zarrow (1951), there is an inability of the anterior pituitary to elaborate sufficient amounts of ACTH in the hypothyroid rat, one would not expect the immediate adrenal response to acute stress (Sayers and Sayers, 1948; 1949; Sayers, 1950) that is obtained after one hour in the cold. Halmi and Bogdanove (1951) have also objected to the hypothesis of Zarrow and Zarrow, basing their disagreement on the finding that no change in ACTH content of the adenohypophysis occurs following thyroidectomy in the rat. Halmi and Bogdanove offered the suggestion that there is an interference with ACTH release, but this explanation also seems untenable in view of the findings described in the present experiment.

Hess (1952) has also found a greater than normal depletion of adrenal ascorbic acid following burn stress in the hypothyroid rat. His results, consistent with the greater than normal depletion of adrenal ascorbic acid and ester cholesterol in the hypothyroid rat found here, might reflect either (1) a more intense than normal cortical response to endogenous ACTH (as suggested by Hess) or (2) a failure of the adrenal cortex to recover stores of these materials after the initial depletion. In either case, it must be emphasized that there is no known manner by which such depletion can be accomplished through stress other than by release of pituitary ACTH. For the first alternative, the early death in cold of the treated animals would mean an absence of positive correlation with the activity

of the pituitary-adrenal axis, whereas the second might explain the failure of survival as an eventual adrenal insufficiency.

That the greater than normal ascorbic acid and ester cholesterol responses in the hypothyroid rat are caused by augmented ACTH release, rather than by impairment of ascorbic acid metabolism is supported strongly by the following evidence.

From the second experiment, it is seen that administration of large doses of ACTH to the thiouracil-treated rat does not increase survival time at low temperature, despite the commencement of such treatment six days before exposure. That the administered hormone was effective in eliciting a cortical stimulation is obvious from the increase in adrenal weight and the thymic atrophy, both associated with continued elaboration of cortical hormone (Crede and Moon, 1940; Moon, 1940; Simpson, Li, Reinhardt, and Evans, 1943).

Ascorbic acid, which according to Dugal and Therien (1949, 1952) protects the normal animal in cold and potentiates the effect of corticotrophin, was likewise without effect in the clipped hypothryoid rat. If a derangement of ascorbic acid metabolism or synthesis were responsible for the magnitude of the adrenal loss of the vitamin in cold it might be expected that administration of ascorbic acid would prevent this and would allow a recovery approaching normal; it is seen that this was not the case.

Perhaps the best evidence for a state of continuous increased activity of the pituitary-adrenocortical system in the hypothyroid rat in cold is found in the experiment involving 96 hours of continuous exposure to 5° C. Here the increased adrenal weight was even greater in the thiouracil-treated rats than in the normal animals, despite the failure of one-third of the treated animals to survive and the poor condition of the other two-thirds at the end of the experiment. It should be noted that the adrenals of these animals had suffered the typical atrophy associated with hypothyroidism at the start of the cold test, and the rats continued to receive the goitrogen during the four days in cold.

Thus the evidence from the experiments reported here strongly supports the view that there is no incompetency of the pituitary-adrenocortical axis in the hypothyroid rat in cold. The immediate response, as reflected by adrenal vitamin C and ester cholesterol depletion, is proof of release of pituitary ACTH, and the thymic involution in the relatively prolonged cold exposures makes unlikely any later breakdown of adrenal function. The inability of either ACTH or potent adrenal cortical extract to prolong survival time of the hypothyroid rat does not appear to allow an explanation for the failure of survival based upon either lack of pituitary ACTH production or release, or inadequacy of adrenocortical response.

The explanation for the adrenal atrophy of the hypothyroid rat at normal environmental temperature that is favored by the data of these ex-

periments is based upon a decreased utilization of cortical hormone in the lowered metabolic state of the thyroid-deficient animal, rather than following from a preoccupation of the anterior pituitary with the production of thyroid stimulating principle (Zarrow and Zarrow), or a disturbance of ACTH release (Halmi and Bogdanove), and is analogous to Sayers' (1950) suggestion for the same condition in chronic inanition.

SUMMARY

The hypothyroid rat subjected to low environmental temperature exhibits a greater than normal adrenal response as measured by depletion of adrenal ascorbic acid and ester cholesterol. Survival time in cold is not prolonged by the administration of ACTH or cortical extract; only thyroxine provides normal resistance. Adrenal hypertrophy and thymic involution following continuous exposure to low temperature are as great in the hypothyroid as in the normal rat, despite which the former fails to survive.

Endocrine factors in the failure of the hypothyroid animal to survive cold exposure, and the adrenal atrophy of such animals at normal temperature, are discussed.

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THE RESPONSE OF THE PITUITARY-THYROID SYSTEM OF THE GUINEA PIG TO LOW ENVIRONMENTAL TEMPERATURE[1]

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ALTHOUGH the influence of low environmental temperature on the functional activity of the thyroid gland has engaged the attention of many investigators, in only a few studies has the interplay of the pituitary-thyroid system in cold exposed animals been assessed by functional criteria in both glands. Activation of the thyroid gland by cold is prevented by hypophysectomy and survival of hypophysectomized animals in the cold is generally poor (Wolf and Greep, 1937, Brolin, 1946). Increase in thyrotrophic (TSH) hormone activity of the pituitaries of rats kept at low environmental temperatures was demonstrated by Kuschinsky (1935) who related this change to the thyroid activation observed after cold exposure. In a noteworthy contribution Brolin (1946) correlated hyperplastic changes in the thyroid with hypertrophy and vacuolization of the anterior pituitary basophils and with heightened TSH levels in the body fluids of cold-exposed rats. The exact mechanism by which the pituitary-thyroid system is activated under conditions of low environmental temperature has not been elucidated. Uotila (1939) and Brolin (1946) demonstrated that anatomic continuity between the pituitary and the hypothalamus was essential for stimulation of the thyroid in cold-exposed animals, from which it was inferred that a neural mechanism influences TSH activity of the anterior pituitary at low temperatures. In contrast Barrnett and Greep (1951) were able to show increased output of thyrotrophic hormone from the pituitary of cold-exposed rats with neural and portal connections from the hypothalamus completely disrupted. These authors interpreted their results as consonant with the hypothesis that the level of the circulating

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target hormone regulates the TSH mechanism of the pituitary. In this regard, the levels of serum protein-bound iodine have been reported to be normal or low in cold-exposed animals (Williams, Jaffe and Kemp, 1949; Ershoff and Golub, 1951; and Rand, Riggs, and Talbot, 1952) despite increased metabolic rates (Mayer and Nichita, 1929; Gelineo, 1934; Ring, 1936; Sellers and You, 1950).

In the present work the relationship between the histophysiologic changes in the thyroid and the adenohypophysis and their relation to the peripheral tissues have been investigated in the cold-exposed guinea pig by measurements of the basal metabolic rate, serum protein-bound iodine, the I^{131} uptake and release by the thyroid gland, thyrotrophic hormone levels in the blood and anterior pituitary and by evaluation of the histocytology of the thyroid and adenohypophysis. The results are considered to indicate that exposure of the guinea pig to low environmental temperature causes an activation of the pituitary-thyroid system, involving increased rates of thyrotrophic and thyroid hormone secretion, which accounts largely for the increased oxidative metabolism in cold. A preliminary report has already appeared (Stevens, D'Angelo, Paschkis, Cantarow and Sunderman, 1953).

MATERIAL AND METHODS

The experimental animals used were 126 adult female guinea pigs of 350–700 gm. body weight receiving unrestricted amounts of a diet of Purina Rabbit Chow Checkers (iodine 5.2 μg./gm.),[6] Purina Omolene (iodine 1.0 μg./gm.)[6] and tap water with a plentiful supply of fresh greens. Guinea pigs were housed together in groups of 6–8, either at normal animal room temperatures of 23° ± 3° C. or in cold room at 7° ± 2° C. Groups of control and cold-exposed guinea pigs were sacrificed at the end of 1 and 6–11 weeks of the experiment.

In a group of 27 guinea pigs both thyroid lobes and as much as possible of the isthmus were removed surgically under ether-urethane anesthesia. In order to reduce the possibility of regrowth of thyroid fragments the animals were injected intraperitoneally with 365–500 microcuries of I^{131} [7] at 1–4 days after operation, and were separated into two groups, one of which was placed in the cold room and the other at room temperature. Both groups were sacrified after 7–8 weeks exposure. At autopsy the tracheal region was inspected macroscopically for thyroid remnants. Suspect tissue was weighed and then prepared for histological examination.

Oxygen consumption was measured by a modification of the closed circuit method of Grad (1952). Two or more measurements of ten minutes duration each were made weekly on at least 8 guinea pigs in any group over the experimental period. Control and cold-exposed animals were tested simultaneously at room temperatures.

The rate of biological decay of radioiodine in the thyroid was determined by an "in vivo" technique recently described (Stevens and D'Angelo, 1954). 25–40 μc. of carrier-free I^{131} were injected intraperitoneally into control guinea pigs maintained at room temperature and into experimental groups immediately before exposure and after 4

[6] Commercial estimate.

[7] I^{131} as NaI in distilled water, obtained from the Atomic Energy Commission, Oak Ridge, Tennessee.

weeks of continuing exposure to cold. Radioactivity over the thyroid region was then measured at 4, 24, 48, and 72 hours after injection and at 3–4 day intervals thereafter for three weeks in the ether anesthetized animal by means of an externally applied gamma sensitive Geiger-Muller tube and scaler. Calculation of theoretical values for extrathyroidal I^{131} of intact guinea pigs was based on measurement of radioactivity of corresponding regions of thyroidectomized animals. The total radioactive counts of the neck region of intact guinea pigs was corrected for "extrathyroidal" counts as well as for isotopic decay. The maximal count obtained in each animal (at 42–72 hours) was expressed as 100%, and successive counts thereafter as a percentage of the maximum.

Whole pituitaries obtained at sacrifice were quickly weighed and fixed in Helly's fluid. Paraffin sections were cut in the horizontal plane at 3–4 micra and stained by Masson's acid fuchsin-Ponceau xylidine-aniline blue method. The periodic acid-fuchsin sulphurous acid (PAS) method of Hotchkiss (1948) was employed on other sections as a means of demonstrating the specific granulation of the basophil cells. Pituitary extracts were prepared by a standard procedure (D'Angelo 1953). Seven to 10 anterior lobes of the pituitary (posterior lobe removed by blunt dissection) were weighed and pooled. The pituitary lobes were homogenized with sand and extracted twice with acidified saline (0.25% glacial acetic acid). After centrifugation the supernatant fluid was assayed for TSH in the stasis tadpole (D'Angelo and Gordon, 1950). TSH was similarly bioassayed in pooled sera obtained from blood withdrawn in groups of animals immediately before sacrifice. The protein-bound iodine of pooled and individual sera was measured by a modification of the Chaney ceric-arsenite reduction method (Barker, Humphrey and Soley, 1951).

RESULTS

All animals placed in the cold suffered a slight initial loss of body weight within the first week of the experiment but resumption of the normal growth rate generally occurred in all experimental groups. Food intake in the cold as measured in a limited number of animals was significantly higher than that at control room temperatures. Table 1 shows the body weights and percentage survival of cold-exposed guinea pigs. About 75% of all intact animals survived exposure of 6–11 weeks at the lowered environmental temperature. Following thyroidectomy 44% of cold-exposed

TABLE 1. BODY WEIGHT AND SURVIVAL OF INTACT AND THYROIDECTOMIZED GUINEA PIGS EXPOSED TO COLD

Group	Exposure to cold (weeks.)	No. of animals	Per cent survival	Body weight (gm.)	
				Initial	Final
Intact	0	8	100	457 ± 11*	458 ± 16*
	1	12	100	458 ± 31	444 ± 49
Intact	0	17	100	437 ± 62	504 ± 105
	6–8	27	77	453 ± 62	481 ± 90
Intact	0	8	100	440 ± 42	465 ± 56
	9–11	17	74	434 ± 71	474 ± 59
Thyroidectomized	0	11	73	489 ± 62	608 ± 80
	7–8	16	44	463 ± 54	609 ± 30

* Mean body weight ± standard deviation.

TABLE 2. PARAMETERS OF PITUITARY AND THYROID FUNCTION IN COLD EXPOSED GUINEA PIGS

Cold exposure (weeks)	Anterior pituitary		Blood TSH conc. μg./ml.	Thyroid mg./100 gm. body wt.[1]	Thyroid I^{131} uptake (%)	
	mg./100 gm. body wt.[1]	TSH conc.[2] μg./mg.			Per 100 mg.	Per gland
0	1.87[2] ±0.01	0.28[3] (0.25–0.38)	0.1[3]	16.0[2] ±0.96	5.0[2] ±0.4	3.5[2] ±0.5
1	1.97 ±0.08	0.22 (0.18–0.28)	0.4 (0.3–0.5)	15.5 ±0.70	4.0 ±0.3	2.7 ±0.4
0	1.87 ±0.18	0.35 (0.28–0.4)	not detectable	15.5 ±1.90	8.0 ±0.2	4.7 ±0.7
6–7	1.80 ±0.08	2.5 (1.6–3.0)	0.16 (0.12–0.18)	11.8 ±0.75	7.6 ±0.3	3.2 ±0.2
0	1.76 ±0.05	0.18 (0.15–0.22)	0.16 (0.13–0.17)	12.7± 0.97	5.3 ±0.9	2.9 ±0.3
9–10	1.90 ±0.08	0.7 (0.5–1.0)	0.4 (0.3–0.5)	11.4 ±0.83	4.7 ±0.5	2.5 ±0.3

[1] Organs related to body weight at sacrifice.
[2] Mean value ± standard error of the mean, based on not less than 7 animals.
[3] Refers to mean concentration of TSH detected (lower and upper limits) in terms of a previously standardized TSH preparation (6–8 Junkmann-Schoeller units per milligram).

guinea pigs survived 7–8 weeks as compared with a 73% survival at higher temperature. Exposure to cold produced no significant change in the weights of the anterior pituitaries of intact guinea pigs but significantly affected the concentration of TSH after 6–10 weeks of refrigeration (Table 2). The concentration of TSH in the anterior pituitaries of intact guinea pigs exposed to cold for one week did not differ significantly from that of the control group but a rise in blood TSH titres was found. After 6–7 weeks of exposure the TSH concentration in the pituitary was increased to about 7 times normal. There were also significantly increased TSH levels in the circulation at this time. The TSH concentration in blood and pituitary was still elevated at the 9–10 week interval, the level in the pituitary being about 4 times that of normal whereas that of the blood was increased 2–3 times.

Histological preparations of pituitaries of cold-exposed guinea pigs showed definite changes as compared with their controls at room temperature. The basophil cells showed a wider distribution and apparent increase in number with a slight diminution of the proportion of acidophils in these regions (Figs. 1, 2). Hyperactivity of the basophils was indicated by their marked enlargement and by greater amounts of cytoplasmic material staining with both Masson and PAS techniques. Golgi negative images

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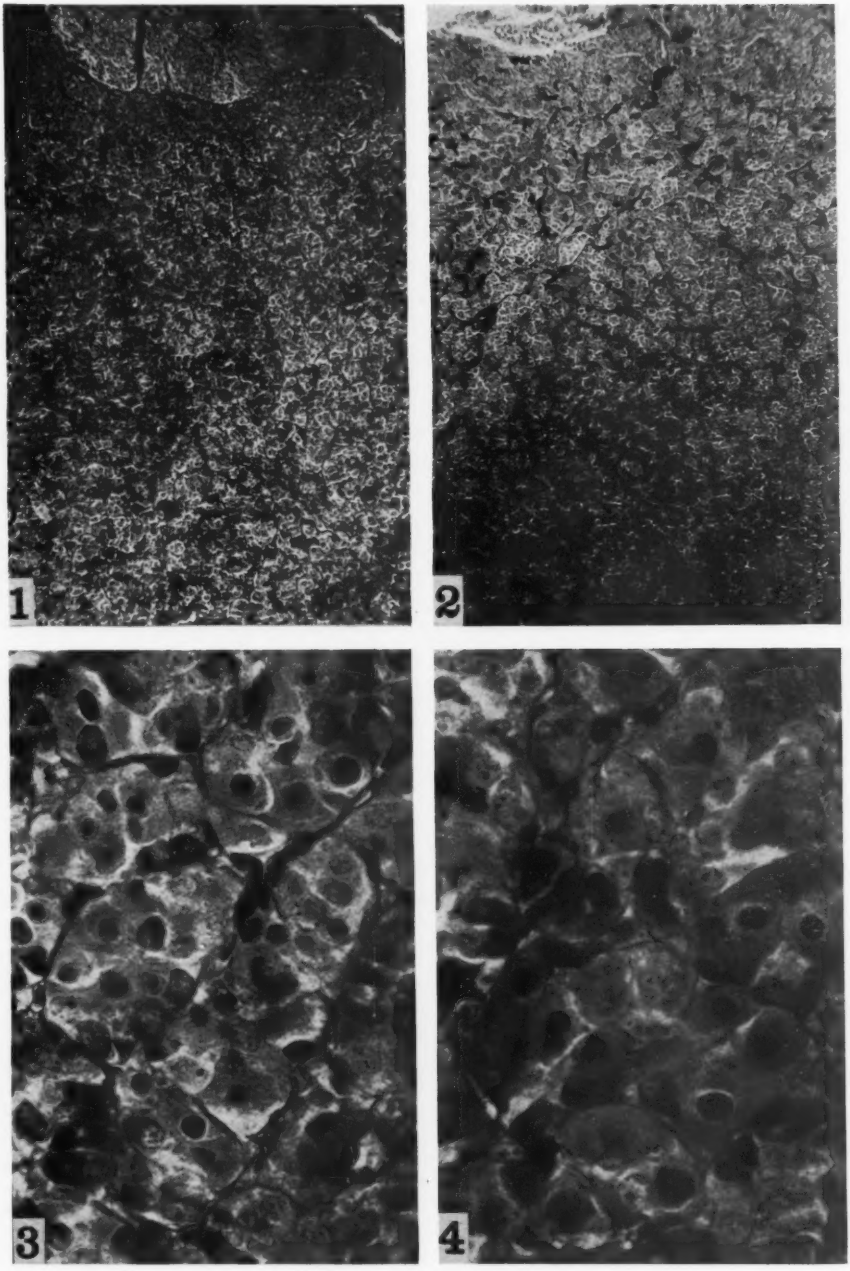
FIG. 1. Normal guinea pig adenohypophysis in the ventromedian region showing general distribution of acidophils (black) and basophils (grey). Intermediate lobe appears above (Helly, Masson, ×100).

FIG. 2. Comparable area showing increased numbers of enlarged basophils (grey, above) with displacement of acidophils (black) after 10 weeks in the cold (Helly, Masson, ×100).

FIG. 3. Normal adenohypophysis containing basophils (grey) with hyperchromatic nuclei (black) and some acidophils (black, elongate) (Helly, Masson, ×300).

FIG. 4. Striking hypertrophy of the basophil cells with deeply stained cytoplasm and enlarged nuclei in the anterior pituitary after 10 weeks of cold exposure (Helly, Masson, ×300).



Photomicrographs of representative sections of thyroid and anterior pituitary in guinea pigs. Panchromatic film and green filter were used.

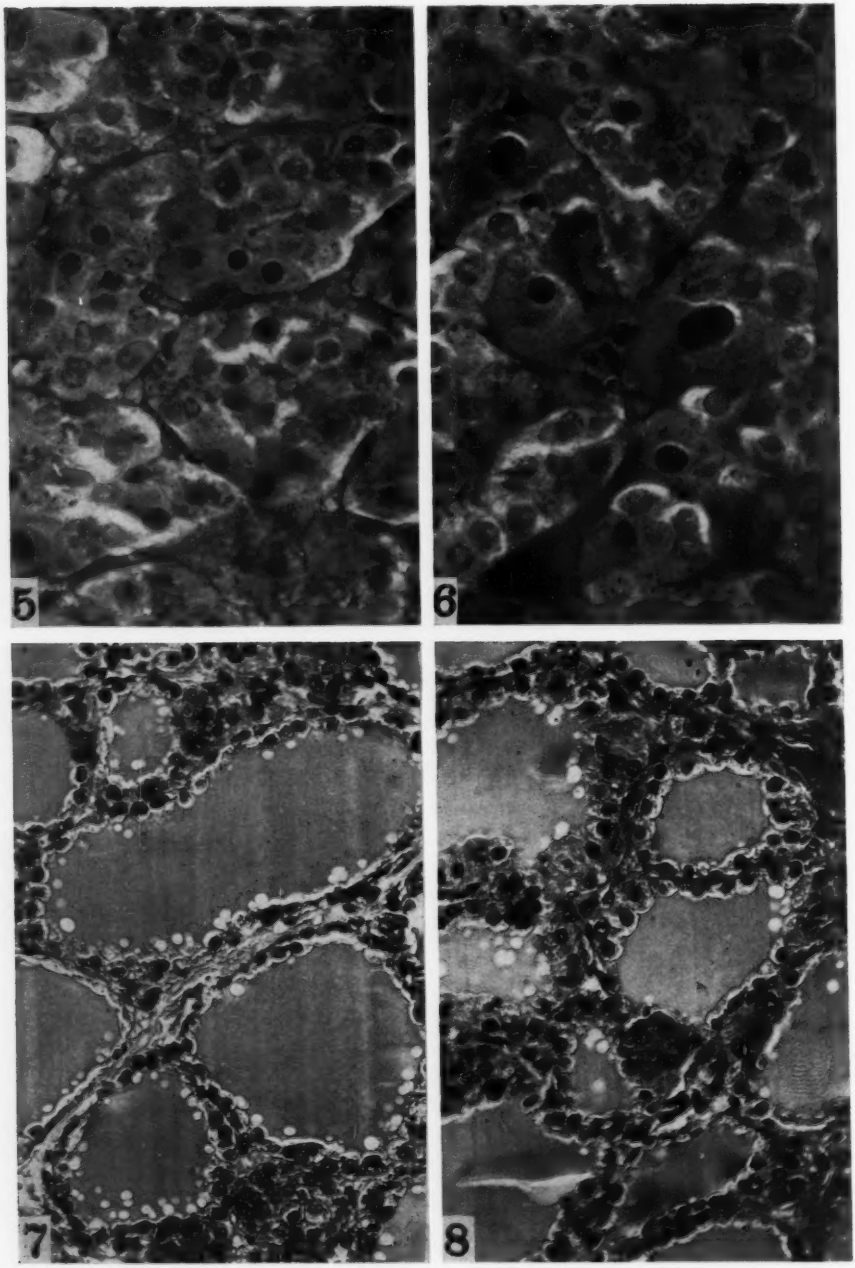


FIG. 5. Normal adenohypophysis after Hotchkiss staining. The basophils (grey, with typically dark nuclei) show moderate amounts of glycoprotein granules in the cyto-

TABLE 3. LOSS OF RADIOACTIVITY FROM THE THYROID OF GUINEA PIGS AT LOW ENVIRONMENTAL TEMPERATURE

Days after zero time[1]	Control[2]	Cold[2]
2	(8)[3]94.0±3.5[4]	(8) 85.4±2.8[4]
4	(8) 85.0±2.9	(7) 79.6±4.7
6	(8) 83.9±2.6	(8) 71.9±3.6
7	(8) 80.4±1.8	(7) 64.6±4.0
10	(12) 73.1±1.9	(13) 57.7±4.1
11	(7) 77.9±1.7	(7) 53.5±4.9
14	(7) 74.2±4.7	(22) 57.5±3.0
15	(12) 71.6±2.8	
18	(7) 59.3±3.1	
19	(12) 61.1±4.3	(6) 44.2±3.5

[1] Zero time: time after injection at which maximal radioactivity (100%) was found in the thyroid.
[2] Control: 23±2° C.; Cold: 7±2° C.
[3] Number of Determinations.
[4] Mean radioactivity in the thyroid as per cent of that at zero time±standard error of the mean.

were prominent in hypertrophied basophils. The nuclei also appeared larger, were frequently polymorphic and contained several prominent nucleoli (Figs. 3–6).

At none of the intervals of cold exposure was the thyroid significantly enlarged (Table 2); however, there were definite signs of histologic activation which could be correlated with the high blood TSH levels as early as one week after exposure. The thyroid of animals in the cold for only one week showed definite morphologic changes indicating heightened release of colloid and elevation of the follicular epithelium. At 9–10 weeks these histological changes were present in more advanced degree (Figs. 7 and 8).

The activation changes in the thyroids of the refrigerated animals were not accompanied by a significant alteration in the 24-hour radioiodine uptake for any of the exposure periods (Table 2). No change was found either in the I^{131} uptake per mg. of wet tissue or in the total radioactivity of the gland. That this was not indicative of the over-all process of iodine collection and secretion occurring in thyroids of animals exposed to cold was demonstrated by the increased rate of biologic decay of thyroidal I^{131}. The graphed relationship of values for loss of radioactivity with time (Table 3)

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plasm. Acidophils and chromophobes show little or no staining reaction (Helly, PAS, ×300).

FIG. 6. Intensification of glycoprotein staining in hypertrophic basophils with enlarged and polymorphic nuclei after 10 weeks exposure to cold (Helly, PAS, ×300).

FIG. 7. Section through the central region of the thyroid in a normal guinea pig (Bouin's picro-formol, H. and E., ×300).

FIG. 8. The histologic response of the thyroid after 6–7 weeks cold exposure illustrating the activation changes in the gland (Bouin's picro-formol, H. and E., ×300).

was approximately linear when plotted on semilogarithmic paper. By extrapolation of the curve, a value of 26 days was obtained for the half-life of radioiodine in the thyroids of guinea pigs maintained at room temperature. It is evident that exposure to cold caused a significant increase in the rate of disappearance of iodine from the thyroid, inasmuch as the biologic half-life was reduced to 13 days. This acceleration of iodine release was of the same magnitude in animals injected immediately before exposure for 1–21 days and in those refrigerated for several weeks before rates of release were measured. Since iodine is known to be released from the thyroid gland principally in the organic form, it was particularly interesting to note that no significant differences existed between the serum PBI levels of cold-

TABLE 4. SERUM PBI AND OXYGEN CONSUMPTION OF INTACT AND THYROIDECTOMIZED GUINEA PIGS EXPOSED TO COLD

Group	Exposure to cold weeks	No. of animals	PBI (μg./100 ml.)	Oxygen consumption	
				cc. O_2/hour/100 gm. body	% change
Intact	0	34	2.0 ± .23[1]	86.6 ± 1.8[3]	
Intact	1	8	2.2[1]	107.2 ± 2.8	+23.8[4]
Intact	3–6	12	1.5 ± .10[1]	112.8 ± 1.5	+30.2
Intact	8–11	15		105.7 ± 2.4	+22.1
Intact	0	28		78.3 ± 1.6	
Thyroidectomized	0	11	1.6 ± .15[2]	65.7 ± 1.4	−16.1
Thyroidectomized[5] (group A)	3–7	6		68.0 ± 2.4	−13.2
Thyroidectomized[5] (group B)	3–7	5		82.7 ± 2.2	+ 5.6
Groups A and B combined		11	1.2 ± .14[2]	75.0 ± 2.3	− 4.2

[1] Grand mean value ± standard error based on determinations in 6 separate groups; 1-week value determined on pooled serum.
[2] Mean value ± standard error based on unpooled samples of 7–8 animals.
[3] Mean values ± standard error for 5–15 animals of group, measured 2–3 times weekly during exposure period.
[4] Percent change from intact control value at room temperature.
[5] Weight range of thyroid remnants; group A, 0–9 mg.; group B, 13.4–67.2 mg.

exposed guinea pigs and that of the controls at the time levels examined (Table 4).

Evidence for a change in the metabolism of peripheral tissues as a reflection of thyroid hormone activity was sought in measurement of the basal metabolic rate. Table 4 shows the oxygen consumption values of intact and thyroidectomized guinea pigs exposed to cold and of control animals maintained at room temperature. It is evident that maintenance of the intact guinea pig in the cold caused a rise (23.8%) in the BMR within the first week of exposure. The increment in oxygen consumption during the 3–6 and 8–11 week intervals was 30.2% and 22.1% respectively. The possibility that low environmental temperature might induce an elevation of the BMR by extrathyroidal means was investigated. The average oxygen consumption measured during the 3rd week through 7th

week after thyroidectomy in animals at room temperature was decreased 16.1% from pre-operative values. In thyroidectomized cold-exposed guinea pigs (groups A and B combined), the corresponding reduction in mean value for oxygen consumption was 4.2%. Although at first glance this would suggest that there is an extrathyroidal component influencing the heat production mechanism in the cold, a consideration of the thyroid remnants found in both thyroidectomized groups of animals indicates otherwise. Despite painstaking dissection and the administration of large doses of radioiodine, functional thyroid remnants were found in the majority of operated animals. Of eight thyroidectomized animals maintained at room temperature, three were free of any demonstrable thyroid tissue. The remaining five had remnants ranging from 5.0–20.0 mg. in weight. Of eleven thyroidectomized cold-exposed guinea pigs, five had remnants weighing 9.0 mg. or less, the remainder bearing remnants weighing 13.6–67.2 mg. The average weight $\pm$ standard error of the mean for both groups was 6.1 ± 2.4 mg. and 17.8 ± 4.6 mg. respectively, for operated animals at warm and cold room temperatures. Histologic examination of all remnants revealed them to be histologically active. It is considered unlikely that the difference in mass of the hypertrophied thyroid tissue found in both groups was fortuitous. Separation of the cold-exposed, subtotally thyroidectomized guinea pigs arbitrarily into two groups on the basis of size of the remnants indicated that those bearing hypertrophied thyroid tissue weighing less than 9 mg. (Table 4, group A) had a mean oxygen consumption of 68.0 ± 1.4 cc. per 24 hours per 100 gm. and those with larger remnants 82.7 ± 2.2 cc. (Table 4, group B). A similar breakdown for animals maintained at room temperature revealed no significant difference in BMR between those totally thyroidectomized and those bearing remnants. When the degree of compensatory hypertrophy of the thyroid tissue is thus taken into account, it would appear that the fall in basal metabolic rate after thyroidectomy is of the same order of magnitude in the cold-exposed animal.

Values for the serum PBI of subtotally thyroidectomized guinea pigs maintained at room temperature for 7–8 weeks after operation although suggestive of a depression in the level, did not show a statistically significant difference from that of the control group. However, exposure of thyroidectomized animals to cold resulted in a slight yet significant drop in the level of serum PBI. Separation of the PBI values into groups on the basis of the size and histologic activation of the thyroid remnants revealed no correlation between the functional status of the remnants and PBI levels, either in the cold-exposed animals or in their controls.

DISCUSSION

Histological activation of the thyroid gland in animals exposed to low environmental temperatures has long been known (Cramer, 1916; Mills,

1918; Kenyon, 1933). Relatively little investigation of thyroid-pituitary interplay under conditions of cold exposure has been made, however, and the few studies reported have been mainly on the rat. The importance of the pituitary in the endocrine response to cold is nevertheless clear. Kuschinsky (1935) described increased content of TSH in the hypophyses of rats exposed at 4° C. for 10 days. Wolf and Greep (1937) demonstrated that hypophysectomy prevented thyroid activation and decreased survival time of rats placed in the cold. The action of cold in altering the thyrotrophic activity of the pituitary was intimated by Starr and Roskelly (1940), who compared the effects of cold and exogenous TSH on histological activation of the rat thyroid. Brolin (1946) demonstrated significant amounts of TSH in the blood and urine of rats after 5–6 weeks exposure to 5–10° C. The rise in TSH titers in refrigerated animals correlated well with hypertrophy and vacuolization of the basophils in anterior pituitary and increased TSH production by the adenohypophysis was inferred. Sellers and You (1950) reported an increase in the TSH content of the pituitary, thyroid enlargement, and increased metabolic rate in rats after 2–13 weeks of exposure to low environmental temperature. Pituitary-thyroid interaction in the guinea pig under conditions of lowered environmental temperatures has received little experimental scrutiny. Borell (1945) observed an increase in thyroid acinar cell height in a group of adult guinea pigs exposed to 4° C. for 10 days but TSH content of the pituitary showed little or no change. TSH levels in the body fluids were not determined. The present study confirms Borell's finding that histological activation of the guinea pig thyroid occurs with relatively brief exposures to cold, and demonstrates further that the response is associated with a significant increase in the blood levels of TSH. With continued exposures, the elevation of blood TSH is maintained and TSH concentrations in the adenohypophysis rise markedly. It is significant that the sequence of changes in TSH content of blood and pituitary of guinea pigs in cold, in which the metabolic demand for thyroid hormone is increased, is similar to that described by D'Angelo (1953) under conditions of inhibited thyroid hormone formation by propylthiouracil. In both instances, blood titres of TSH are elevated before TSH stores in the hypophysis are substantially increased.

The cyto-physiologic alterations found in the adenohypophyses of guinea pigs at lowered temperatures were sufficiently marked and definite to permit critical analysis of these morphologic changes in terms of the problem of cellular localization of TSH production. The cytologic activation of the anterior pituitary basophils either with cold or in propylthiouracil-induced goitrogenesis (D'Angelo, 1953) and the unmistakable association, in each instance, with thyroid hyperplasia and TSH increase in blood and hypophysis strongly implicate the basophil as the producer of TSH. Baillif (1938) and Brolin (1946) have similarly interpreted the increase in numbers, size and vacuolization of basophils in cold exposed rats as re-

flecting augmentation of thyrotrophic hormone secretion. Although two types of basophilic cells in the guinea pig pituitary comparable with the thyrotrophs (betas) and gonadotrophs (deltas) of the rat (Purves and Griesbach, 1951A, 1951B; Halmi, 1950, 1952), have not been delineated, it it considered significant that the hypertrophied basophils in the former species show considerable increase in glycoprotein granulation when TSH content of the anterior pituitary is also high. It is also pertinent that the TSH content of the rat adenohypophysis is normally considerably greater than that of the guinea pig (D'Angelo, 1953; D'Angelo, Stevens, Paschkis and Cantarow, 1953) and that glycoprotein staining granules in the thyrotrophs disappear with massive discharge of TSH from the rat pituitary (Halmi, 1952; Purves and Griesbach, 1951B). Although further analysis of these PAS staining basophils in the guinea pig with the PAS, aldehyde-fuchsin positive basophils of the rat cannot be made at present, the possibility must not be dismissed that the enlarged basophils appearing in the hypophysis of guinea pigs under conditions of cold or PTU-induced goitrogenesis are homologous with the thyrotrophs of the rat.

The absence of significant change in the 24-hour I^{131} uptake by the cold-stimulated thyroid does not contradict measurements of other parameters which point indisputably to activation of the pituitary-thyroid system. In general, reports of radioiodine uptake by the thyroids of cold-exposed rats are not in accord. In short-term exposures (2–8 days) to cold, thyroidal radioiodine collections 1–4 hours after I^{131} injection were increased (Catz, Rawi and Geiger, 1953; Leblond, Gross, Peacock and Evans, 1944), or decreased (Williams, Jaffe and Kemp, 1949). For similar periods of exposure, the 18–30 hour thyroidal I^{131} uptakes were unchanged (Catz *et al.*, 1953) or decreased (Leblond *et al.*, 1944). Longer exposure to cold (4 weeks) led to thyroidal radioiodine uptakes which were significantly increased at the 2 hour but not at the 30 hour interval (Leblond *et al.*, 1944). All of these results were generally interpreted as indicating accelerated accumulation of radioiodine by the thyroid in cold-exposed animals. In this connection, the maximal uptake of I^{131} in the guinea pig thyroid, as measured by the "in vivo" method, was achieved earlier in the cold-exposed animals than in controls. The customary 24-hour I^{131} uptake by the gland, however, was well within the time required for maximal accumulation of radioactivity (48 hours). Extrathyroidal factors may operate to reduce the radioiodine collection by the thyroid; possibilities in this regard are increased renal excretion of iodide attending the known activation of the pituitary-adrenal system in the present study (Stevens, D'Angelo, Paschkis, Cantarow and Sunderman, 1953) or reduction in specific activity of the circulating iodide consequent to the ingestion of greater amounts of dietary I^{127}, reflecting increased food consumption in the cold. Measurement of the rate of release of radioiodine from the thyroid apparently reveals more accurately the functional status of the gland

in refrigerated animals. The shorter biological half-life of thyroidal radioiodine of cold-exposed guinea pigs suggests that the rate of thyroxine secretion is increased at low temperatures. This view is in accord with those held by Dempsey and Astwood (1943), and Rand, Riggs and Talbot (1952), who found that relatively greater amounts of thyroid substance were required to prevent goitre in cold-exposed animals receiving antithyroid agents.

The elevation of oxygen consumption in the cold-exposed animal in the presence of normal or slightly lowered serum PBI levels bears on two important questions regarding the physiologic adjustments made by animals at low environmental temperatures; namely, 1) the role of extrathyroidal factors in heat production, 2) the mechanism underlying thyroid-hypophyseal interplay in the cold. Ring (1936) has shown that in rats elevation of the BMR by cold is mediated largely if not exclusively by an extrathyroidal mechanism; however, the presence of the thyroid gland apparently is essential for survival in the cold, inasmuch as Leblond and Eartly (1952) were not able to maintain completely thyroid-deficient animals at even the moderately lowered temperature of 16° C. beyond 4 weeks. Although the thyroidectomy experiments of the present study were largely in the exploratory phase, it would seem that the increased BMR which attends cold exposure in the guinea pig could be prevented in large part by subtotal thyroidectomy. It is significant, furthermore, that in operated animals in which oxygen consumption remained elevated in cold, the functional thyroid remnants were appreciably larger. In animals completely thyroidectomized or with small remnants, a decrement in BMR occurred which approximated that in the thyroidectomized animals maintained at room temperature. Elevation of the BMR in intact refrigerated animals with normal or lowered serum PBI levels has been observed frequently in the rat (Rand, Riggs and Talbot, 1952; Ershoff and Golub, 1951; Williams, Jaffe and Kemp, 1949). Inasmuch as the increased oxygen consumption in the refrigerated guinea pig depended upon the presence of the thyroid in which the rate of hormonal secretion was accelerated, it is likely that the nearly normal levels of serum PBI in the circulation result from correspondingly increased utilization or disposal in the tissues. This would be compatible with the generally accepted concept of anterior pituitary-thyroid interplay (D'Angelo, 1954) as excessively high levels of circulating thyroid hormone would conceivably suppress TSH secretion from the pituitary. Thus, in the cold-exposed animal heightened thyroid hormone secretion and its activity at the periphery are maintained without impairing the thyrotrophic mechanism. It is also significant that in the subtotally thyroidectomized, cold-exposed animal the serum PBI levels were somewhat lower than those at room temperature despite larger functional remnants, and that in these refrigerated animals, the basal metabolic rate approached that of the normal intact

condition. It would appear, therefore, that the increased oxidative metabolism of the cold-exposed guinea pig may involve a commensurate utilization or disposal of thyroid hormone. It cannot be stated with certainty, however, whether increased oxidative metabolism and peripheral utilization of the thyroid hormone precede activation of the pituitary-thyroid system in our experiments. Examination of serum PBI levels and the rates of disappearance of labeled thyroid hormone of totally thyroidectomized animals at both room and lowered environmental temperatures would be informative in this regard.

SUMMARY AND CONCLUSIONS

The pituitary-thyroid system in the adult female guinea pig was studied during a 1–11 week period of exposure to lowered environmental temperature ($7 \pm 2°$ C.). Histological activation of the thyroid and increased blood titres of TSH were found after one week in the cold. Longer exposures (6–11 weeks) induced a significant elevation in TSH concentration in the anterior pituitary. This was accompanied by a pronounced increase in the number of hypertrophied basophil cells containing increased amounts of glycoprotein granules (Hotchkiss-McManus staining).

Level of serum protein-bound iodine, thyroid-body weight ratios, and 24-hour I^{131} uptake by the thyroid were not appreciably altered by exposure to lowered environmental temperature. The rate of release of radioiodine from the thyroid was approximately doubled, however, and significant increases in oxygen consumption were found throughout the entire exposure period. The elevation in BMR by cold could be largely abolished by thyroidectomy.

Analysis of the experimental data indicates that heat production in the cold-exposed guinea pig is maintained primarily by an activation of the pituitary-thyroid system in which secretion of thyrotrophic hormone and thyroid hormone is augmented.

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ANALYSIS OF THE MODIFYING EFFECT OF DIETARY IODINE LEVELS ON THE THYROIDAL RESPONSE OF HYPOPHYSECTOMIZED RATS TO THYROTROPHIN[1]

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VANDERLAAN and Caplan (1954) and Halmi (1954b) have shown that the response of the thyroidal iodide concentrating mechanism of hypophysectomized rats to exogenous thyrotropin (TSH) is influenced by the iodine content of the diet. The same dose of TSH was found to be more effective in raising the thyroid: serum radioiodide gradient when iodine intake was low than when when it was high.

A priori this phenomenon is amenable to three interpretation: a) the stable iodide of the diet, being the substrate of the concentrating mechanism, could diminish the gradient of the subsequently administered radioiodide by overloading the iodide concentrating mechanism, whose capacity is limited; b) prolonged elevation of serum iodide levels *per se* may actually depress the iodide concentrating mechanism and/or inhibit its responsiveness to TSH, or c) a rise in the level of serum iodide may exert such an influence indirectly by enhancing the concentration of organic iodine-containing compounds in the thyroid. However, since the serum iodide levels of the rats maintained on the high iodine regimens in the studies of VanderLaan and Caplan (1954) and Halmi (1954b) could not have been sufficiently high to depress the radioiodine gradient by overloading the iodide concentrating mechanism,[2] the first hypothesis can be dismissed. The present study was undertaken to determine which of the remaining two explanations for the effect of iodine intake on the response of the iodide concentrating mechanism to TSH is correct.

Groups of hypophysectomized rats were maintained on a low, a medium or a high iodine diet and treated with a standard dose of TSH alone or in

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[2] In the study of VanderLaan and Caplan (1954) the high iodine diet contained 5 mg. of KI/kg.; in the experiment of Halmi (1954b) the iodine concentration of the high iodine regimen was 12 mg./kg. Taurog and Chaikoff (1946) reported serum iodide levels of 3.8 and 8.3 μg.% in rats fed diets containing 5 and 10 mg. of iodine/kg. respectively. Depression of the thyroid: serum radioiodide gradient through overloading of the iodide concentrating mechanism requires serum iodide levels of over 100 μg.% (Halmi, 1954a).

conjunction with propylthiouracil injections. The results of the study indicate that, under the conditions of the experiment, variations in iodine intake failed to affect the iodide concentrating mechanism if organic binding of iodine in the thyroid was blocked by the goitrogen.

MATERIALS AND METHODS

The experiments were carried out on hypophysectomized, male Sprague-Dawley rats (150–180 gm.) obtained from the Hormone Assay Laboratories, Chicago, Ill. Five days after the operation groups of animals were placed on the following regimens: a) a Remington diet,[3] containing <0.1 mg. of iodine per kg., or the same diet fortified with b) 1.8 mg., or c) 18 mg. of iodide (as NaI) per kg. Five days later the rats on each of the three diets were divided into two groups, one of which received daily subcutaneous injections of 1 USP unit of thyrotrophin (TSH) (20 mg. of USP Thyrotropin Reference Substance) for 10 days, and the other, the same dose of TSH accompanied by daily subcutaneous injections of propylthiouracil (10 mg. in suspension form) for 10 days. All animals were sacrificed 24 hours after the last injection. Thyroid: serum radioiodide concentration determinations and follicular cell height measurements were performed as described in earlier papers (Halmi *et al.*, 1953; Halmi, 1954b).

RESULTS

The results of the experiments are summarized in Table 1.

Thyroid weights did not appear to be significantly affected, whether iodine intake was varied or propylthiouracil (PTU) was administered together with the thyrotrophin (TSH). Thyroid follicular cell heights were greater in the low iodine group which received PTU plus TSH than in the animals on the same diet which received TSH alone. However, PTU had no significant effect on the cell height response to TSH in rats that were not iodine deficient. Although in the goitrogen plus TSH treated rats the thyroid cell height tended to diminish as the iodine content of the diet increased, such was not the case in the rats given TSH alone. In fact, among

TABLE 1. EFFECT OF DIETARY IODINE LEVELS ON THE THYROIDAL RESPONSE OF HYPOPHYSECTOMIZED RATS RECEIVING THYROTROPHIN (TSH) ALONE OR IN CONJUNCTION WITH PROPYLTHIOURACIL (PTU)

Treatment	Diet	No. rats	Thyroid weight, mg.	Mean acinar cell height, μ	Thyroid: serum radioiodide gradient
TSH alone	Low I	10	9.2 ± .67*	9.9 ± .25	122.7 ± 10.9
	Medium I	11	9.4 ± .68	10.9 ± .29	67.6 ± 5.4
	High I	10	9.3 ± 1.00	10.8 ± .39	32.6 ± 2.7
TSH plus PTU	Low I	18	8.8 ± .39	11.5 ± .30	166.2 ± 14.5
	Medium I	14	9.6 ± .61	10.3 ± .37	182.6 ± 17.8
	High I	16	7.9 ± .40	9.6 ± .30	160.8 ± 21.0

* Mean ± standard error.

[3] "Low Iodine Test Diet," Nutritional Biochemicals Corporation, Cleveland, Ohio.

the latter animals those on the iodine deficient diet showed the smallest cell height.

In the rats which received TSH alone, the response of the thyroidal iodide concentrating mechanism, as indicated by the thyroid: serum iodide concentration (T/S) ratio, declined sharply as the iodine content of the diet increased. The T/S ratio in the animals given TSH plus PTU, which was at a higher level than even that of the iodine deficient TSH treated rats, failed to be influenced by the amount of iodine in the diet. Consequently, the augmentation by PTU of the response of the iodide concentrating mechanism to TSH (Halmi *et al.*, 1953; VanderLaan and Caplan, 1954; Halmi and Spirtos, 1954) was much more striking when iodine intake was high than when it was low.

DISCUSSION

The differences between the mean follicular cell heights of the various experimental groups of this study were not sufficiently clear-cut to justify attempts at interpretation. It is of interest, however, that propylthiouracil (PTU) did not augment the effect of TSH on thyroid cell height when iodine intake was adequate, whereas such a potentiating effect of the goitrogen had been observed in a previous investigation (Halmi and Spirtos, 1954). This discrepancy may be due to the fact that the more prolonged treatment with TSH in the present study resulted in maximal hypertrophy of the follicular epithelium whereby further enhancement of the response by PTU was precluded.

The magnitude of the thyroid: serum iodide concentration (T/S) ratio, which reflects the performance of the iodide concentrating mechanism, was inversely proportional to the iodine intake in the rats which received the standard dose of TSH alone, but was conspicuously unaffected by dietary iodine levels when organic binding of iodine was blocked with PTU. One may argue that the T/S ratio was maximal even in those rats treated with TSH plus PTU which were on the high iodine diet, and that further elevation of the gradient could thus not take place when the iodine intake was reduced. However, T/S ratios of over 400 have been previously observed in hypophysectomized rats which received TSH and PTU (Halmi and Spirtos, 1954), whereas in the present experiments the highest mean gradient was only approximately 180; this tends to negate the above argument.

The fact that iodine intake, under the conditions of this experiment, failed to modify the response of the iodide concentrating mechanism to TSH unless organic binding of iodine in the thyroid was permitted, is in agreement with a hypothesis evolved in an earlier paper (Halmi and Spirtos, 1954). On the basis of the studies of Halmi *et al.*, (1953), VanderLaan and Caplan (1954), and Halmi and Spirtos (1954) the idea was advanced that the thyroidal iodine concentrating mechanism is controlled by

two antagonistic factors: TSH, its stimulant, and an intrinsic thyroidal organic iodine-containing principle (possibly thyroid hormone), its depressant. Taurog and Chaikoff (1946) have shown that total iodine and thyroxine concentrations in the thyroid rise with increasing iodine intake until an upper limit is reached. The following interpretation of the findings reported in this paper thus appears reasonable:

In the rats receiving the standard dose of TSH alone, the response of the iodide concentrating mechanism varied with the level of dietary iodine because the latter determined the concentration of the hypothetical inhibitor of the iodide concentrating mechanism within the thyroid. In the rats treated with TSH and PTU, iodine intake had no effect on the T/S ratios because dietary iodine could not become organically bound in the thyroid and therefore could not enhance the concentration of the depressant in the gland. Serum iodide levels *per se* apparently do not affect the T/S ratio until they become sufficiently high to overload the iodide concentrating mechanism.

SUMMARY

In hypophysectomized rats treated with a standard dose of thyrotrophin (TSH) the response of the thyroidal iodide concentrating mechanism was inversely proportional to the dietary iodine levels. Variations in iodine intake, however, failed to affect the reaction of the iodide concentrating mechanism to TSH when propylthiouracil was also administered. These findings are interpreted as supporting the concept that an organic iodine-containing compound within the thyroid, possibly thyroid hormone itself, antagonizes the stimulating action of TSH on thyroidal iodide collection.

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EFFECT OF CORTICOTROPIN ON CELLULARITY AND MITOSIS IN THE RAT BONE MARROW, SPLEEN AND THYMUS[1]

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PROLONGED administration of corticotropin or cortisone to rats decreases the bone marrow cellularity (Baker and Ingle, 1948; Thiersch *et al.*, 1952). The reduction in lymphatic tissue mass produced by adrenocortical steroids has been attributed to lymphocytolysis and mitosis inhibition (Dougherty, 1953).

The present report is concerned with a comparative study of the effects of corticotropin on the cellularity and mitosis in the rat bone marrow, spleen and thymus as determined by chemical methods. Since desoxyribonucleic acid (DNA) is relatively constant per somatic nucleus, the DNA phosphorus content of the tissue furnishes a quantitative estimate of its cellularity. The DNA synthesis as measured by the rate of incorporation of radiophosphorus into the DNAP, is a sensitive index of mitosis. Injections of corticotropin for 20 days resulted in a decrease in cellularity and mitosis in the three organs studied, the effects being greatest on the thymus and least on the bone marrow.

METHODS

Intact, male rats of the Sprague-Dawley strain, 112–120 days old and weighing 300–400 grams, were divided into litter mate pairs to serve as control and treated animals. The rats were tube fed a modification of the high protein diet reported by Ingle, Prestrud, Li, and Evans (1940)[3] to prevent or diminish the negative nitrogen balance and weight loss of adult rats treated with corticotropin. The diet was administered twice daily for nine days before and throughout the experimental period. Rats between 300 and 350 grams received 20 ml. feedings and those between 350 and 400 grams received 23 ml. feedings. All animals were weighed daily.

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[2] Markle Scholar in Medical Science.

[3] DIET: Celluflour, 90 gm.; Osborne-Mendel salt mixture, 20 gm.; Wheat germ oil, 5 gm.; Synthetic Vit. K (Abbott Lab.), 0.1 gm.; Dried yeast (Pabst), 50 gm.; Powdered Egg Albumin, 250 gm.; Powdered Casein (Labco), 150 gm.; Mazola oil, 75 gm.; Cod liver oil, 5 gm.; Water to make 1500 ml.

Purified corticotropin[4], 20 U.S.P. units per ml., suspended in 20% gelatin, 30% propylene glycol, 1% sodium phosphate, and 0.5% phenol was injected subcutaneously into the interscapular region of the treated group in doses of 10 units per kilogram at twelve hour intervals (6–8 units daily). The control animals received 0.35 ml. of a mixture containing 14% gelatin, 15% propylene glycol, and 0.5% phenol at twelve hour intervals.

At the third, tenth, and twentieth days after beginning injections of corticotropin or the control mixture, animals, which had fasted for twelve hours, were injected intraperitoneally with approximately 2 microcuries of carrier free $NaH_2P^{32}O_4$ per 100 gm. body weight. Four hours later they were sacrificed under ether anaesthesia by section of the abdominal aorta and vena cava. The spleen, thymus, and both adrenal glands were removed and weighed. Methods for obtaining samples of bone marrow from the femurs and tibias, the separation of acid soluble phosphorus (ASP), ribonucleic acid phosphorus (RNAP), and DNAP, and estimation of the P content and P^{32} incorporation into these fractions have been described (Rambach *et al.*, 1952, 1953). The results of these estimations have been analyzed statistically for probability of a significant difference between the control and treated groups at each interval by the Student "t" analysis (Fisher, 1950).

Estimation of the hemoglobin content and leukocyte count were made on samples of tail blood obtained at the time of sacrifice. Differential counts of 200 cells were performed on films of the peripheral blood and bone marrow.

RESULTS

Control animals maintained a stable weight, whereas those receiving corticotropin lost approximately 10% of their original body weight by the

[4] Kindly supplied by Dr. E. E. Hayes of Armour Laboratories, Chicago, Illinois.

TABLE 1. MEAN TOTAL CONTENT AND RELATIVE SPECIFIC ACTIVITIES FOR THE PHOSPHORUS FRACTIONS OF BONE MARROW, SPLEEN, AND THYMUS

	Group	No. rats	Total phosphorus content*			Specific activity†		
			ASP	RNAP	DNAP	ASP	RNAP/ASP	DNAP/ASP
SPLEEN	*3 day*							
	Control	4	.790 ± .072	.527 ± .063	.972 ± .123	.474 ± .046	.304 ± .056	.182 ± .066
	ACTH	4	.769 ± .072	.371 ± .142	.835 ± .198	.461 ± .076	.326 ± .051	.154 ± .077
	10 day							
	Control	3	.605 ± .140	.509 ± .058	.852 ± .055	.526 ± .074	.342 ± .013	.179 ± .113
	ACTH	3	.532 ± .090	.420 ± .186	.674 ± .115	.465 ± .045	.303 ± .028	.148 ± .040
	20 day							
	Control	6	.854 ± .050	.650 ± .193	.983 ± .338	.500 ± .054	.302 ± .017	.228 ± .094
	ACTH	4	*.405* ± .060	*.265* ± .057	*.356* ± .074	.508 ± .069	.284 ± .051	.088 ± .065
THYMUS	*3 day*							
	Control	2	.384 ± .052	.301 ± .043	.906 ± .106	.449 ± .041	.329 ± .014	.107 ± .002
	ACTH	2	.160 ± .125	.124 ± .106	.292 ± .276	.360 ± .028	.325 ± .073	*.044* ± .006
	10 day							
	Control	3	.248 ± .041	.205 ± .025	.566 ± .057	.606 ± .016	.313 ± .039	.109 ± .018
	ACTH	3	*.090* ± .022	*.064* ± .013	*.078* ± .022	.535 ± .040	.325 ± .069	.130 ± .045
	20 day							
	Control	6	.237 ± .067	.241 ± .092	.483 ± .167	.540 ± .053	.287 ± .033	.133 ± .011
	ACTH	4	*.088* ± .020	*.045* ± .074	*.041* ± .042	.469 ± .133	.475 ± .154	.119 ± .060
BONE MARROW	*3 day*							
	Control	4	1.132 ± .063*	.941 ± .031*	1.453 ± .044*	.420 ± .066	.451 ± .028	.435 ± .037
	ACTH	4	1.023 ± .155	.853 ± .064	1.326 ± .020	.382 ± .011	.503 ± .041	.457 ± .061
	10 day							
	Control	3	1.155 ± .134	1.060 ± .229	1.602 ± .326	.511 ± .022	.388 ± .050	.416 ± .020
	ACTH	3	.942 ± .049	.747 ± .138	1.100 ± .158	.434 ± .025	.471 ± .047	.499 ± .057
	20 day							
	Control	6	1.133 ± .117	.944 ± .051	1.332 ± .089	.476 ± .037	.414 ± .045	.426 ± .062
	ACTH	4	*.857* ± .084	*.652* ± .058	*.860* ± .096	.463 ± .086	.464 ± .084	.480 ± .040

* Phosphorus values for bone marrow are expressed as mg. per gm. wet weight.
† Percentage of P^{32} injected/mg. P in fraction. Italics indicate significant differences from control animals (P value >0.98).

twentieth day. During the course of the experiment, adrenal weights of the control group increased slightly, but the corticotropin stimulated adrenal glands were heavier at all periods and after 20 days were double the weight of the controls. In the experimental animals there was a fall in weight of the spleen to 88, 87 and 66% and the thymus to 51, 60 and 55% of the controls at 3, 10 and 20 days respectively.

The content and relative specific activity of the ASP, RNAP and DNAP fractions of the bone marrow, spleen and thymus are given in Table 1. Since the total marrow weight cannot be estimated, phosphorus content of

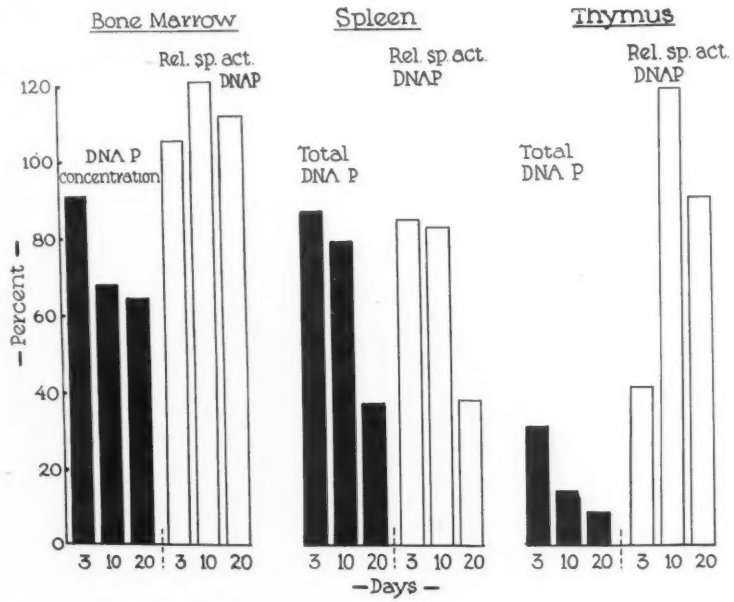

FIG. 1. Effect of corticotropin on the content and relative specific activity of the DNAP in the bone marrow, spleen and thymus.

the three fractions from this organ is expressed as mg. P per gm. wet weight of tissue. Changes in these values are assumed to parallel those of the entire bone marrow. In the spleen and thymus, the ASP, RNAP and DNAP contents are expressed as total mg. P per organ and reflect changes both in concentration and organ weight. The four hour incorporation of P^{32} is presented as the specific activity (per cent of injected P^{32} per mg. P) for the ASP fraction and the relative specific activity for the RNAP and DNAP fractions (specific activity RNAP or DNAP/specific activity ASP). The DNAP contents and relative specific activities expressed as per cent of the appropriate control values are shown graphically in Figure 1.

The relative specific activity is related to the percentage of cells dividing

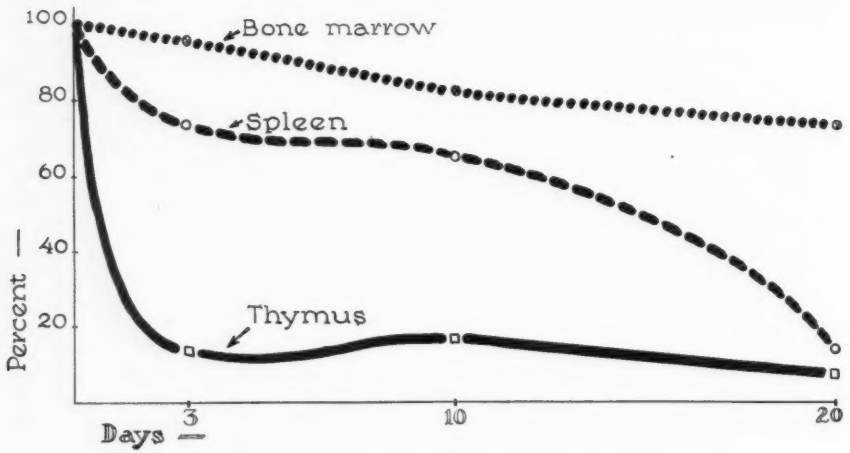

FIG. 2. Effect of corticotropin on the total mitotic activity of the bone marrow, spleen and thymus.

and is not necessarily a measure of the total mitotic activity of an organ. An index of the total mitotic activity or new cell formation may be obtained by multiplying the relative specific activity of the DNAP by the DNAP content. These values have been computed per unit weight of bone marrow and for the total spleen and thymus and are recorded graphically as percentage of the control values in Figure 2.

Peripheral blood values and bone marrow differential counts are given in Table 2.

TABLE 2. EFFECT OF CORTICOTROPIN ON MEAN PERIPHERAL BLOOD VALUES AND BONE MARROW DIFFERENTIAL COUNTS

	3 days		10 days		20 days	
	Control	ACTH	Control	ACTH	Control	ACTH
PERIPHERAL BLOOD						
No. Rats	4	4	3	3	6	4
Hemoglobin (gm./100 cc.)	13.9	14.7	13.7	15.2	15.2	16.8
WBC per mm.^{3} ×1000	29.8	19.3	30.4	25.1	25.8	19.9
Lymphocytes per mm.^{3} ×1000	14.5	11.4	15.5	13.2	11.6	7.3
Eosinophils per mm.^{3} ×1000	1.18	.29	.43	.47	.45	.05
BONE MARROW						
No. Rats	3	4	3	3	5	4
Neutrophilic granulocytes %	50	45	50	64	50	52
Eosinophilic granulocytes %	6	5	4	4	5	1
Erythrocytic series %	12	8	15	9	20	15
Other cells %	32	42	31	23	25	32

DISCUSSION

The bone marrow cellularity as measured by the DNAP content fell to 65 per cent of the control value after 20 days of corticotropin administra-

tion. There was no significant change in the DNA synthesis per unit number of cells (relative specific activity of DNAP). However, the mitotic activity per gram of marrow, which is presumably representative of the total bone marrow, decreased to 75% of the control by the end of the experimental period.

The total cellularity of the spleen declined slowly at first, but at the end of the experiment was reduced to about one-third of the control value. Mitosis rate, as well as total mitotic activity, dropped sharply between the tenth and twentieth day of treatment. Inhibition of mitosis appears to be a factor in the splenic involution produced by corticotropin.

Changes in the thymus occurred more rapidly than in the spleen. Both the weight and cellularity were markedly reduced in 3 days and at 20 days the total cellularity was about a tenth of the control. Mitosis rate was initially depressed and then returned to normal at 10 and 20 days. Total mitotic activity remained below 20% of the control values between the third and twentieth day of treatment. It is possible that the presence of a normal mitosis rate in the markedly involuted thymus is due to persistence of reticuloendothelial and other cells resistant to the effect of corticotropin.

The RNAP contents and relative specific activities in the bone marrow and spleen in general paralleled the DNAP values. In the thymus the high ratio of the RNAP to DNAP is further evidence of an increased percentage of cells rich in cytoplasm such as reticuloendothelial cells and young lymphocytes.

The slightly higher hemoglobin values observed in the treated animals could have been due to a more rapid maturation and delivery of erythrocytes or to hemoconcentration associated with the 10% reduction in body weight. The depression of lymphocytes and eosinophils is evidence of adrenocortical stimulation. The only change noted in the bone marrow differentials was an equivocal decrease in cells of the erythrocytic series.

The above experiments show the depressing effects of prolonged corticotropin administration on cellularity and total mitotic activity in the bone marrow, spleen and thymus. The effects are least marked on the bone marrow which contains little or no lymphatic tissue, and greatest on the thymus which is primarily a lymphoid organ. The spleen, which contains some myeloid tissue in the rat, falls in an intermediary position. The reduction in cellularity following adrenocortical stimulation can be caused by inhibition of mitosis, cell destruction or increased cell delivery. The relative importance of each mechanism cannot be evaluated from these studies, but a decrease in total mitotic activity has been clearly demonstrated. Destruction of cells capable of mitosis could explain this phenomenon.

Dougherty and White (1945) have shown that the involution of lymphatic tissue following an injection of ACTH is associated with degenerative changes in the lymphocytes and cessation of mitosis of the small and medi-

um sized lymphocytes. Hull and White (1952) observed a 20% decrease of P^{32} uptake by the DNA of mouse lymph nodes and thymus during the first few hours following a single injection of corticotropin. No inhibition was noted in the spleen. The latter authors consider these findings to reflect in part the inhibition of mitosis of lymphocytes by the hormone.

SUMMARY

Corticotropin gel in doses of 10 units per Kg. was administered daily to tube fed adult rats and determinations made of the cellularity and mitosis in the bone marrow, spleen and thymus after 3, 10 and 20 days. The desoxyribonucleic acid phosphorus content of these organs was used as a measure of cellularity and the rate of incorporation of P^{32} into the DNAP as an index of mitosis. After 20 days the cellularity in the bone marrow was reduced to 65, the spleen to 33 and the thymus to 10 per cent of the control values. Total mitotic activity was depressed to 75, 15 and 7 per cent of the controls respectively.

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SERUM POLYSACCHARIDE CHANGES IN THE RAT FOLLOWING HYPOPHYSECTOMY AND THE ADMINISTRATION OF PITUITARY GROWTH HORMONE[1]

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AN ELEVATION of protein bound polysaccharides occurs in the serum of patients with cancer, various of the collagen diseases, tuberculosis, and sarcoidosis (Seibert, Seibert, Atno and Campbell, 1947 and Shetlar *et al.*, 1950). An elevation of serum polysaccharide is also found in late pregnancy (Shetlar *et al.*, 1950). Fever and processes incidental to tissue destruction (Seibert *et al.*, 1947) or to tissue proliferation (Shetlar *et al.*, 1949) have been postulated to be associated with these findings. Injuries to experimental animals including bacterial infection, turpentine abscesses, intraperitoneal talc insufflation, surgical procedures (Shetlar *et al.*, 1949) epidermal thermal injury (Knobloch *et al.*, 1952) and pyrogen induced fever (Shetlar *et al.*, 1953) produce a rise of serum polysaccharide.

The mechanisms by which these changes are produced present intriguing avenues of investigation as such elevations may reflect chemical aspects fundamentally related to these conditions. As an abundance of work as reviewed by Selye (1950) would indicate that endocrine influences are of importance in the above states, investigation of hormonal influence on the serum polysaccharide levels would seem to be of interest. The present study was initiated in an effort to explore the influence of the anterior pituitary gland on the protein polysaccharide ratio.

METHODS

Nonglucosamine (hexose) polysaccharide was determined by the tryptophan method of Shetlar, Foster and Everett (1948). Total serum protein was determined and fractionated with 26% Na_2SO_4 as previously described (Shetlar, Shetlar *et al.*, 1950). The fraction soluble in 26% sodium sulfate was assumed to be largely albumin and is designated hereafter as the "albumin fraction." Seromucoid polysaccharide was determined by the tryptophan method after isolation of the seromucoid fraction by the method of Winzler and Smyth (1948).

Rats of the Holtzman strain were used. Two groups of males and two groups of fe-

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[1] This work was supported in part by a grant from the American Cancer Society on the recommendation of Committee on growth of the National Research Council.

males were studied in order to determine sex differences. Initial weights of the different groups also varied, being 120–150 and 200–250 gm. for the male groups and 60–75 and 100–125 gm. for the female groups. Hypophysectomy was done through the parapharyngeal approach. Growth hormone was obtained from two different sources.[2]

RESULTS

Average results obtained from studies of 46 normal rats, 64 hypophysectomized without treatment, and 56 animals given growth hormone after hypophysectomy are summarized in Table 1. Hypophysectomized rats

TABLE 1. SUMMARY OF SERUM POLYSACCHARIDES IN NORMAL AND HYPOPHYSECTOMIZED RATS WITH AND WITHOUT GROWTH HORMONE

	No. of rats	Bound Hexose[1] of				
		Total protein		Albumin		Seromucoid
		Mg. %	% of T. protein	Mg. %	% of albumin	Mg. %
Normal rats	46	164 ± 4	2.59 ± .04	16 ± .4	0.70 ± .02	8 ± .4
Hypophysectomized	64	143 ± 2*	2.43 ± .03*	13 ± 1.3	0.68 ± .02	8 ± .4
Hypophysectomized +Growth Hormone	56	163 ± 3	2.73 ± .04†	15 ± 1.3	0.83 ± .03†	9 ± .4

[1] Figure following each average is the standard error for the series.
* Significantly lower than the control group $P < .01$.
† Significantly higher than the control group $P < .01$.

were found to have significantly lower serum hexose polysaccharide levels than sham operated or normal controls. Hypophysectomized rats injected with growth hormone developed serum polysaccharide levels significantly higher than their uninjected controls. The albumin polysaccharide concentration of hypophysectomized rats was slightly, but not significantly reduced while those of the hypophysectomized group given growth hormone were significantly higher in this polysaccharide fraction. No differences in seromucoid polysaccharide were noted between the various groups.

Results of serial determinations of total serum polysaccharides in one experiment are depicted graphically in Figure 1, illustrating lower levels in hypophysectomized rats without treatment, while the hypophysectomized rats given growth hormone develop higher than normal levels. These higher levels are maintained even after the animals cease to grow upon administration of growth hormone.

One group of 7 female hypophysectomized rats was given growth hormone and compared to a similar group of 7 rats without growth hormone. After an appropriate time the two groups were interchanged. Results of this study are depicted in Figure 2. In this group good agreement of serum polysaccharides with growth response is demonstrated. No appreciable dif-

[2] Kindly supplied by Dr. M. S. Raben, New England Center Hospital, Boston and Dr. Irby Bunding, Armour Laboratories, Chicago.

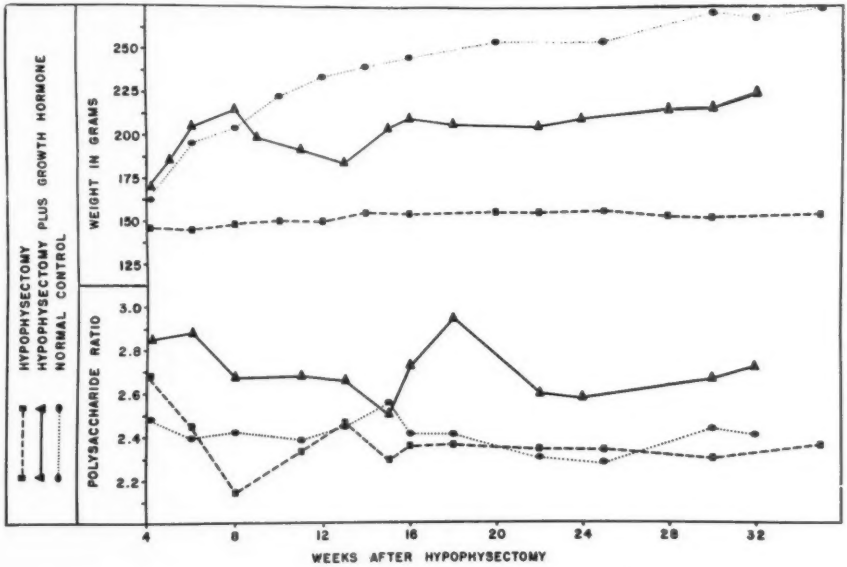

FIG. 1. The effects of hypophysectomy (■- - - -■), and of hypophysectomy followed by daily subcutaneous injections of 0.2 to 0.4 mg. of growth hormone (▲———▲) on the serum polysaccharide-protein ratio of male rats. Levels of intact rats of similar age are charted for comparison over the same period of time (●· · · ·●). Growth curves of the respective groups are plotted above the polysaccharide curves. Results based on serial studies of 12 hypophysectomized untreated, 18 hypophysectomized, growth hormone treated, and 10 intact animals. All animals were females.

ferences between the two growth hormone preparations were noted in either growth rate or effect on polysaccharide levels.

EFFECT OF TRANSPLANTED TUMORS

Growth of the transplanted Walker 256 tumor results in elevation of serum polysaccharide (Shetlar, Erwin, and Everett, 1950). In order to test

TABLE 2. EFFECT OF TRANSPLANTED WALKER 256 TUMOR ON THE SERUM POLYSACCHARIDE OF HYPOPHYSECTOMIZED AND NORMAL RATS

	No. of rats	Tumor size	Bound Hexose of				
			Total protein		Albumin		Seromucoid
		mm.²	Mg. %	% of T. protein	Mg. %	% of albumin	Mg. %
Hypophysectomized							
Before	6	—	145	2.43	15	0.73	6
After 18 days		520	174	3.37	28	2.17	28
Normal							
Before	12	—	149	2.28	17	0.74	7
After 12 days		2104	146	3.51	32	2.04	30

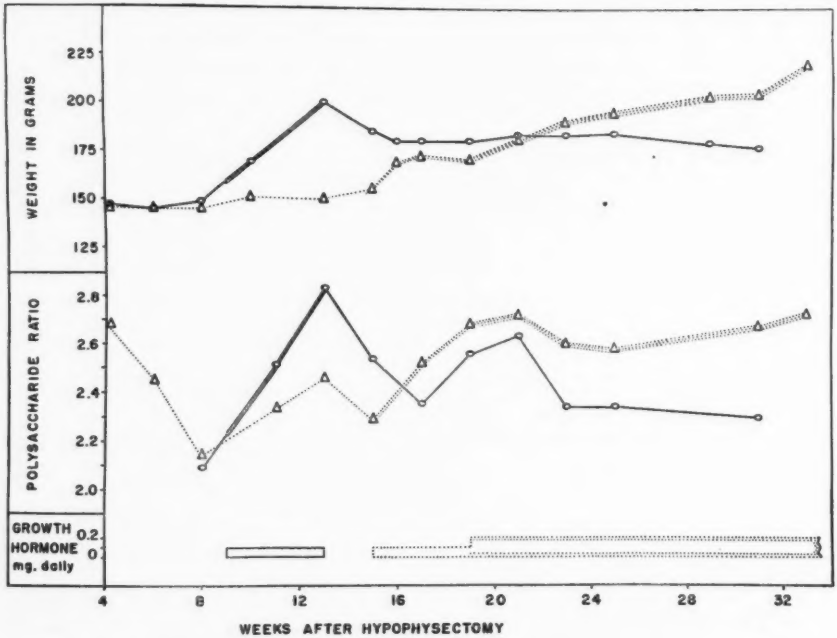

FIG. 2. The effects of growth hormone on the serum polysaccharide-protein ratio of female rats. Solid and dotted lines represent two different groups. Double lines indicate the daily subcutaneous injection of 0.2 mg. of growth hormone. Triple line indicates the daily subcutaneous injection of 0.4 mg. of growth hormone. Growth curves of each group are plotted above the polysaccharide curves. Results based on studies of 7 female rats in each group.

the ability of hypophysectomized animals to respond in a similar way, Walker 256 tumors were transplanted by means of a No. 15 hypodermic needle under the loose skin of the abdominal wall of 6 hypophysectomized

TABLE 3. EFFECT OF TURPENTINE ABSCESSES ON SERUM POLYSACCHARIDES OF HYPOPHYSECTOMIZED AND NORMAL RATS

	No. of rats	Bound Hexose of				
		Total protein		Albumin		Seromucoid
		Mg. %	% of T. protein	Mg. %	% of albumin	Mg. %
Hypophysectomized						
Before turpentine	6	147	2.62	12	0.61	6
3 days after turpentine		194	4.02	39	3.14	31
7 days after turpentine		221	3.79	36	2.55	26
Normal						
Before turpentine	7	174	2.85	18	0.88	7
3 days after turpentine		210	3.94	44	3.10	26
7 days after turpentine		194	3.49	24	1.45	16

rats. A control series of 12 normal rats of approximately this same age were similarly innoculated with the tumor at the same time in order to directly compare normal and hypophysectomized rats. Results are given in Table 2. As previously reported by McEuen and Thompson (1933) tumor growth was much slower in the hypophysectomized rats. However, elevations of the serum polysaccharides were of the same magnitude in both groups.

The effect of turpentine abscesses was also investigated. One half cc. of turpentine solution was injected intramuscularly into the thigh flexor muscles of 7 normal and 6 hypophysectomized rats. Results are presented in Table 3. Elevated polysaccharides of the same magnitude occurred in both groups.

DISCUSSION

It may be seen that hypophysectomy is followed by lowering of the serum polysaccharide level in the rat. Administration of growth hormone to these operated animals results in an elevation somewhat above normal levels. None of this elevation is due to a rise in seromucoid. This situation is similar to that noted in pregnancy in which total serum polysaccharide becomes elevated but no change occurs in the seromucoid fraction (Shetlar *et al.*, 1950). An elevation of polysaccharide found in the albumin fraction occurs in hypophysectomized rats treated with growth hormone; elevation of this fraction also occurs in pregnancy. This would seem to support the possibility that a carbohydrate rich fraction occurring in the albumin of serum (as separated with 26% Na_2SO_4) may be correlated with growth or proliferative processes. However, the changes in P R found after administration of growth hormone to hypophysectomized rats are less pronounced than the elevation noted in pregnancy, cancer, arthritis, and many other conditions. Furthermore, in some cases elevated serum polysaccharide levels persisted after rats ceased to grow upon further administration of growth hormone. Two different preparations of growth hormone reported to have disproportionate amounts of diabetogenic hormone produced the same effect. Thus it would seem that this factor also is not immediately responsible for the effect obtained. Injections of beef albumin at the same protein level were made to test the effect of nonspecific protein. These injections were without appreciable effect on the serum polysaccharide levels.

Hypophysectomy apparently does not impair the ability of the rat to produce protein bound carbohydrate under conditions of stress; in the presence of a transplanted tumor the elevation of serum polysaccharide was approximately the same in these animals, as compared to normal animals. Similarly, subcutaneous injection of turpentine resulted in elevations of the same magnitude in hypophysectomized and intact animals. It is indicated, therefore, that although the pituitary has some influence on serum polysaccharide levels, this effect is not a direct one and other factors are operative.

SUMMARY

Hypophysectomy in rats resulted in a significant drop in the serum polysaccharide-protein level. Administration of either of two growth hormone preparations to these animals at levels of 0.2–0.8 mg. per day resulted in polysaccharide levels significantly higher than those of intact control animals. The seromucoid fraction was not altered by either hypophysectomy or growth hormone.

Transplanted tumors or turpentine abscesses in hypophysectomized rats resulted in elevated serum polysaccharide and seromucoid levels indicating that hypophysectomy does not impair the ability of the rat to produce protein bound carbohydrate under conditions of stress.

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AN EXPERIENTIAL FACTOR INFLUENCING THE EFFECTIVENESS OF TESTOSTERONE PROPIONATE IN ELICITING SEXUAL BEHAVIOR IN MALE GUINEA PIGS[1]

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CONSIDERABLE evidence supports the view that the patterns of sexual behavior displayed in response to hormonal stimulation are related to the character of the soma or substrate on which the hormones act rather than to the amount of hormone, provided a certain threshold quantity is present (Young, Dempsey, Myers and Hagquist, 1938; Boling, Young and Dempsey, 1938; Young and Fish, 1945; Grunt and Young, 1952, 1953; Riss and Young, 1954). More recently it has been shown for the male guinea pig that patterns of sexual behavior are formed as the result of an interaction of experiential and genetic factors. Contact with other animals plays an important role in the acquisition of the mature copulatory pattern, but the amount of contact that is required varies depending on the genetic strain to which the individual belongs (Valenstein, Riss and Young, 1955). The suggestion follows that contact with other animals in influencing the development of patterns of sexual behavior somehow changes an organism's response to hormonal stimulation. Investigation of the hypothesis was undertaken by means of a relatively simple experiment.

MATERIALS AND METHODS

Inbred Strain 2 male guinea pigs (Valenstein, Riss and Young, 1954; Riss, 1955) were used because the development of sexual behavior in males of this strain is so conspicuously dependent on contact with other animals (Valenstein, Riss and Young, 1955). Nineteen males (the socially raised animals) were confined with their mothers and siblings for 25 days. They were then weaned and confined with 5 females of the same size until day 73. At this time they were isolated and seven weekly tests of sexual performance (Young and Grunt, 1951; Valenstein, Riss and Young, 1954) were begun on day 77. Seventeen males (the isolated males) were isolated from all other animals the day of birth except that they were alone with their mothers for 25 days. On day 77 seven weekly tests of their sexual performance were begun. Thirteen of the socially raised males and 10 of the isolated males were castrated on day 120, after the completion

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of the seventh test. The others were retained as intact controls. Toward the end of a 10-week period each of the castrates was given three tests for the purpose of ascertaining whether the level of behavior had regressed to the baseline commonly reached by the castrated male guinea pig (Grunt and Young, 1952). Following these tests, 5 of the isolated animals and 7 of the socially raised males received 25 μg. of testosterone propionate[2] per 100 gm. body weight daily for 10 weeks; 5 of the isolated males and 6 of the socially raised animals received 100 μg. The androgen was dissolved in sesame oil and was administered by intraperitoneal injections. The level of performance was compared with that of the controls which were tested weekly during the last seven weeks of the terminal 10-week period.

RESULTS

Prior to castration the pattern of behavior of the socially reared males was remarkedly different from that of the isolated males. Only 1 of the 17

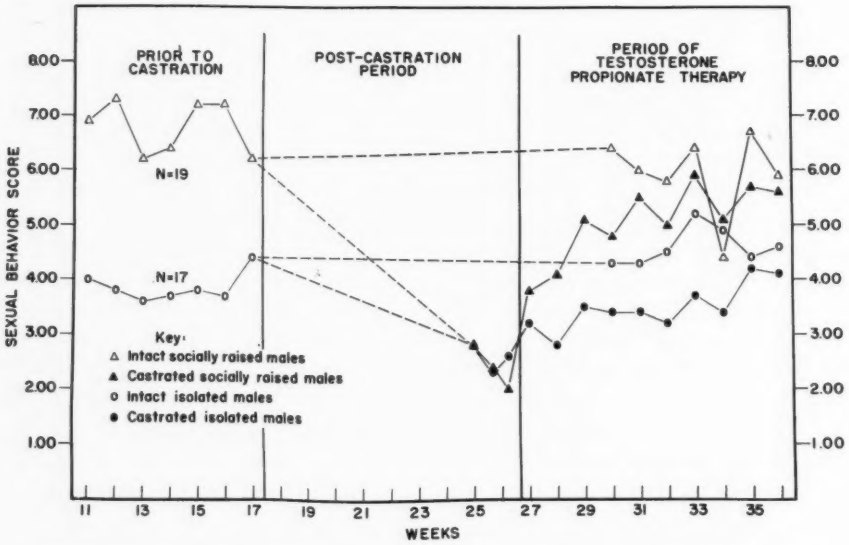

FIG. 1. Sexual behavior scores of socially raised and isolated males prior to castration, in the post-castrational period and during the period of testosterone propionate therapy.

isolated males intromitted and ejaculated, whereas 16 of the 19 socially raised males did so consistently. This difference which is significant ($P < .01$) is reflected in the sexual behavior scores (fig. 1) which are based on the maturity as well as the quantity of sexual behavior exhibited and on the length of the interval between the beginning of the test and ejaculation (Young and Grunt, 1951). Regardless of the social situation in which the animals were raised, castration was followed within 10 weeks by a de-

[2] Testosterone propionate was supplied by Ciba Pharmaceutical Products, Inc.

crease in strength of sex drive to the same baseline. By this time none of the males was having intromissions or ejaculations, but a few of the socially reared animals occasionally mounted a female. The administration of testosterone propionate restored the strength of drive to nearly the level that characterized each group of males prior to castration. The circumstance that the average test score of the intact, socially reared controls during the 34th week was considerably below the usual level for these animals was due to an unexplainable poor performance by 1 of the 6 males.

A somewhat different approach to the data is given in Table 1 in which the percent of males exhibiting mounting, intromission and ejaculation

TABLE 1. PER CENT OF MALE GUINEA PIGS RAISED IN ISOLATION AND IN A SOCIAL SITUATION EXHIBITING MOUNTING, INTROMISSION AND EJACULATION 1) PRIOR TO CASTRATION AND 2) 10–20 WEEKS AFTER CASTRATION WHEN TESTOSTERONE PROPIONATE WAS ADMINISTERED

			Per cent mounting	Per cent intromitting	Per cent ejaculating
Isolates	Castrates ($N=10$)	Precastrational tests	30	10	10
		Tests during hormonal therapy	30	10	10
	Intact Controls ($N=5$)	Preliminary tests	60	0	0
		Retests	40	0	0
Social	Castrates ($N=13$)	Precastrational tests	100	85	85
		Tests during hormonal therapy	92	83	75
	Intact Controls ($N=6$)	Preliminary tests	100	100	83
		Retests	100	83	67

during the precastrational period and during the period of androgen therapy is summarized. A relatively large percent of the socially reared males exhibited these behavioral units in the precastrational period and during the period with replacement therapy this percentage was approximately restored. On the other hand, only 1 of the 10 isolated males which were castrated intromitted or ejaculated during the precastrational testing period. On the retests with replacement therapy this same male was the only animal in the group to achieve a level of behavior as high as intromission. However they are analyzed, therefore, the results show clearly that the effectiveness of testosterone propionate as a stimulant of sexual behavior depends to a significant extent on the experiential background of the male. As the data reveal, administration of the hormone to males in the social and isolated groups was followed by an increase in the vigor with which they pursued the female, but only those males which had previously given evidence of possessing an organized pattern of sexual behavior were able to copulate successfully.

It will be recalled that some animals received 25 μg. of testosterone propionate per 100 gm. body weight daily and others 100 μg. The results from the animals that were socially raised or isolated have been analyzed by the

method of *paired replicates* (Wilcoxon, 1949). The socially raised castrates receiving 100 μg. of testosterone propionate did slightly better than those receiving 25 μg., but the difference is not statistically significant. However, the isolated castrates receiving 100 μg. did significantly better than the isolated males receiving 25 μg. The fact that there was a significant difference in the isolate group and not in the social group may perhaps be explained by the greater variability in the scores of animals that have intromissions and ejaculations.

DISCUSSION

The experiment described above provides an additional link in the investigation of a problem that has long been of interest to the student of hormones and behavior. As we have noted, much of the difference between individuals can be attributed to differences in the reactivity or responsiveness to gonadal hormones rather than to the quantity of hormone. Two factors, the genetic background and contact with other animals, seemed most likely to be responsible for these differences. Riss and Young (1953) have demonstrated that the genetic background of an animal is an important determinant of its response to androgenic stimulation, but the present experiment is believed to provide the first demonstration that an experiential factor can influence an organism's response to androgenic stimulation. It is of interest that Lehrman (1954) has reached a similar conclusion with respect to the ability of prolactin to elicit feeding behavior in ring doves. Injections of this hormone were found to be necessary for the eliciting of feeding behavior, but they were effective only with doves which had fed young before.

The fact that the isolated males receiving 100 μg. of testosterone propionate per 100 gram body weight did significantly better than those receiving 25 μg. requires comment. The results appear to contradict those obtained by Grunt and Young (1952, 1953) who reported that differences in sexual performance were not related to amount of androgen, provided a threshold quantity was surpassed. Their data, however, were obtained from a genetically heterogeneous stock. It is possible that the threshold for Strain 2 males is above 25 μg. If so, the difference between the 25 and 100 μg. groups would be understandable and consistent with the interpretation given previously.

Studies of the responsiveness of the seminal vesicles to testosterone propionate support the opinion expressed by Grunt and Young (1952). The growth response of the seminal vesicles of the rat was no greater when 1.0 mg. of this hormone was injected daily than when 0.5 mg. was given (Moore and Price, 1938). The epithelial cell height and the content of alkaline phosphatase in the seminal vesicles of castrated guinea pigs were not different in animals receiving 12.5 and 100 μg. of testosterone propionate per 100 gm. body weight daily (Grunt, 1954). The seminal vesicles

removed from 8 of the castrated, treated males used in this investigation have been studied (Sinks, unpublished data). Those from 4 males receiving 100 μg. of testosterone propionate are slightly heavier than those from 4 males receiving 25 μg., but the difference is not significant and a proportional relationship is not suggested.

SUMMARY AND CONCLUSIONS

An experiment was undertaken to determine whether the experiential background of the male guinea pig could influence the effectiveness of testosterone propionate in eliciting sexual behavior.

Thirteen socially reared and 10 isolated males of the inbred Strain 2 were castrated after it had been ascertained by standard tests with females that the level of sexual behavior of the socially raised animals was significantly higher than that of males raised in isolation. After 10 weeks, when the animals had regressed to a base line of sexual activity, testosterone propionate was administered. Each group returned to nearly the level of behavior that had been characteristic of the precastrational period. The socially raised males which had previously given evidence of possessing an organized pattern of sexual behavior exhibited intromissions and ejaculations. None of the isolated males ejaculated and only one animal had an intromission. It is concluded that the experiential background can do much to determine the character of the behavior that is displayed in response to testosterone propionate.

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THE RESPONSE OF ENDOMETRIAL INTRAOCULAR IMPLANTS FOLLOWING TOTAL AND REGIONAL X-IRRADIATION OF THE HOST[1,2]

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PREVIOUS observations indicate that exposure to ionizing radiation is capable of altering the endocrine integration essential for cyclic reproductive phenomena in the female. The evidence consists largely of observed abnormalities in the estrous rhythm of guinea pigs (Genther, 1931), rats (Freed *et al.*, 1948; Leitch *et al.*, 1949), and mice (Bischoff *et al.*, 1944). Other indications of endocrine disturbances in reproduction are suggested by the reports of Mandel (1935) that precocious sexual maturity occurs in young rats following exposure to x-rays and of Schmidt (1937) that decreased estrogen excretion during estrus occurs in guinea pigs after local irradiation of the ovary.

The present study was undertaken to determine whether total body irradiation is capable to modifying the growth of endometrial tissue in the rabbit, and, if possible, to establish whether such modifications could be attributable to changes in the level of endogenous hormone or to changes in the sensitivity of the target tissue to hormone stimulation. The data of the experiments reported herein show that total body exposure to x-rays elicits a pattern of response in intraocular endometrial implants which differs appreciably from that observed in nonirradiated animals. This response appears to reflect changes in the hormone levels of the host and is dependent, at least in part, upon the region exposed.

METHODS

Young adult dark-eyed female Dutch rabbits, 5–5½ months of age, were used. The animals, kept in an air-conditioned room (60–65° F.), were housed individually in solid sided cages to prevent pseudo-pregnancy. Food (Purina Laboratory Rabbit Pellets) and

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[2] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official, or as reflecting the views of the Navy Department. This work was supported, in part, by funds provided by an ONR contract, U.S. Navy Department.

water were available at all times except during the 24 hours prior to intraocular implantation when food was withheld. A total of 81 females were prepared with autologous intraocular endometrial implants after the method of Benjamin, Belt, and Krichesky (1940), following subtotal hysterectomy. At the time of the operation, gross observations were made of the number and type of follicles present in each ovary as well as the size, vascularity, and motility of the uterus. With few exceptions, two implants were placed in each eye (Fig. 1). Two hundred and eighty-eight implants (approximately 90%) remained functional to furnish the data reported herein.

A minimum of $2\frac{1}{2}$ weeks post-operation was allowed for the implants to become physiologically organized. Then, a series of photographs were taken. This was done at

FIG. 1. Two endometrial implants in the anterior chamber of the eye of an adult female rabbit.

the same time of day in order to avoid the complication of diurnal variation reported by Jacobsen (1944). The areas of the implants were determined over a 10 to 14 day interval by a method using planimeter measurements[3] of the projected negatives (Krichesky *et al.*, 1943). If the area of a given implant did not show fluctuations greater than $\pm 6\%$, the implant was considered to be stabilized, and the average of the 10 to 14 day period was designated as 100% for that particular implant. Subsequent changes in area were plotted upon coordinate paper in terms of per cent area increase or decrease.

[3] This photographic method, using planimeter measurements for determining the area of intraocular implants, was demonstrated to be reproducible to $\pm 2\%$ (Jacobsen, 1944).

Following irradiation, all experimental animals and their respective nonirradiated controls were photographed daily for 5 days and then at 2 to 4 day intervals for an additional 15 to 20 days.

In each series to be described the animals were distributed as equally as possible according to age, weight, and litter among the treated and control groups. The rabbits were placed in thin-walled Lucite boxes and exposed to a horizontal x-ray beam. Four[4] animals were irradiated at each exposure. The position of the boxes in the radiation field was such that the dose rate variation along the central axis of the boxes was less than $\pm 5\%$ of the mean dose rate. A dose of 800 r was employed which was equivalent to an LD-50/30 day dose for total body irradiation. The radiation factors were: 250 KVP; 15 ma.; filter, 0.5 mm. Cu plus 1.0 mm. Al; HVL 1.5 mm. Cu; target distance, 50 in.; dose rate of 14 r/min. as measured in air with a Victoreen ionization chamber placed inside the Lucite box at the site occupied by the center of the rabbit's body during the exposure. Dose measurements were accurate to $\pm 5\%$ of the stated dose. The animals were rotated 180° at half exposure intervals in order to equalize the dose to both sides of the body. The controls were sham irradiated and were manipulated in a manner similar to that of their irradiated partners.

At the conclusion of the intraocular experimental series, an additional group of 8 animals was exposed to 800 r whole body irradiation and sacrificed at various intervals thereafter in order to examine the genital tract *in situ*. Similar observations were made of the genital tract in 8 nonirradiated animals.

RESULTS

Implant growth in nonirradiated animals: Twenty-three animals with a total of 82 functional implants were used as controls. The area of most implants in the sham irradiated animals fluctuated about the the baseline mean ($\pm 10\%$) in a noncyclic manner, previously described (Jacobsen, 1944). In an occasional animal the implants showed a gradual increase in area during the period observed. The mean growth of the implants in the sham irradiated controls of 4 separate experiments is depicted in Figure 2.

Implant growth following 800 r total body irradiation: The effect of total body irradiation upon intraocular endometrial growth was studied in 4 experiments comprising a total of 22 irradiated animals with 74 functional implants. Each experiment included a sham irradiated group for comparison. The data revealed a consistent difference in the behavior pattern of implants in the totally exposed animals compared to that observed in their sham irradiated controls, irrespective of the fact that the experiments were conducted at different seasons of the year (x-rayed in January, May, August, and October, respectively). In no instance did the controls exhibit the surge of growth observed in whole-body-irradiated animals immediately following exposure (Fig. 2). A generalized pattern was discernible when the response of all of the implants in the totally irradiated groups was considered simultaneously (see Fig. 3A). During the first 48 hours following irradiation, an appreciable increase in implant area occurred.

[4] In a few instances requiring exposure of 3 animals, a paraffin phantom was substituted for the fourth animal.

The greater proportion of this increase took place during the initial 24 hours and attained a peak within 48 hours in the majority of the animals. The implant area was maintained, but to a lesser degree, for a period of 8 to 10 days post-irradiation.

The implants in irradiated animals that died during the observation period exhibited a pattern of response corresponding to that obtained in irradiated survivors except during the terminal 48 hours. Usually a precipitous decrease in area (approximately 20%) took place during the 2 days prior to death. Since this decrease obviously was associated with morbidity the data for the last 48 hours in the case of nonsurvivors were discarded.

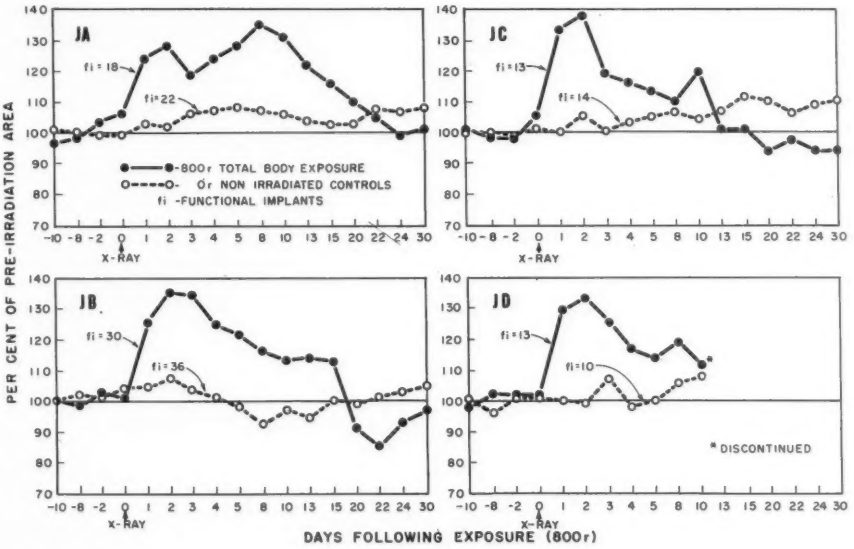

FIG. 2. Changes in area of intraocular endometrial implants in 4 series of adult female rabbits following exposure to 800 r whole body irradiation.

Implant growth following regional body irradiation: From the results described above it seemed pertinent to determine whether they were attributable to the direct effect of ionizing radiation upon the target tissue or whether they were mediated through general systemic reactions involving the endocrines. For this purpose various types of regional body irradiation were employed. Shielding various regions of the body was accomplished as follows:

1) *Head:* physically immobilizing the animal in a tight fitting perforated Lucite box covered on all sides with a lead plate (0.125 in. in thickness) over the area to be shielded (see Fig. 3H).
2) *Body (excluding head):* as in (1). See Figure 3B.

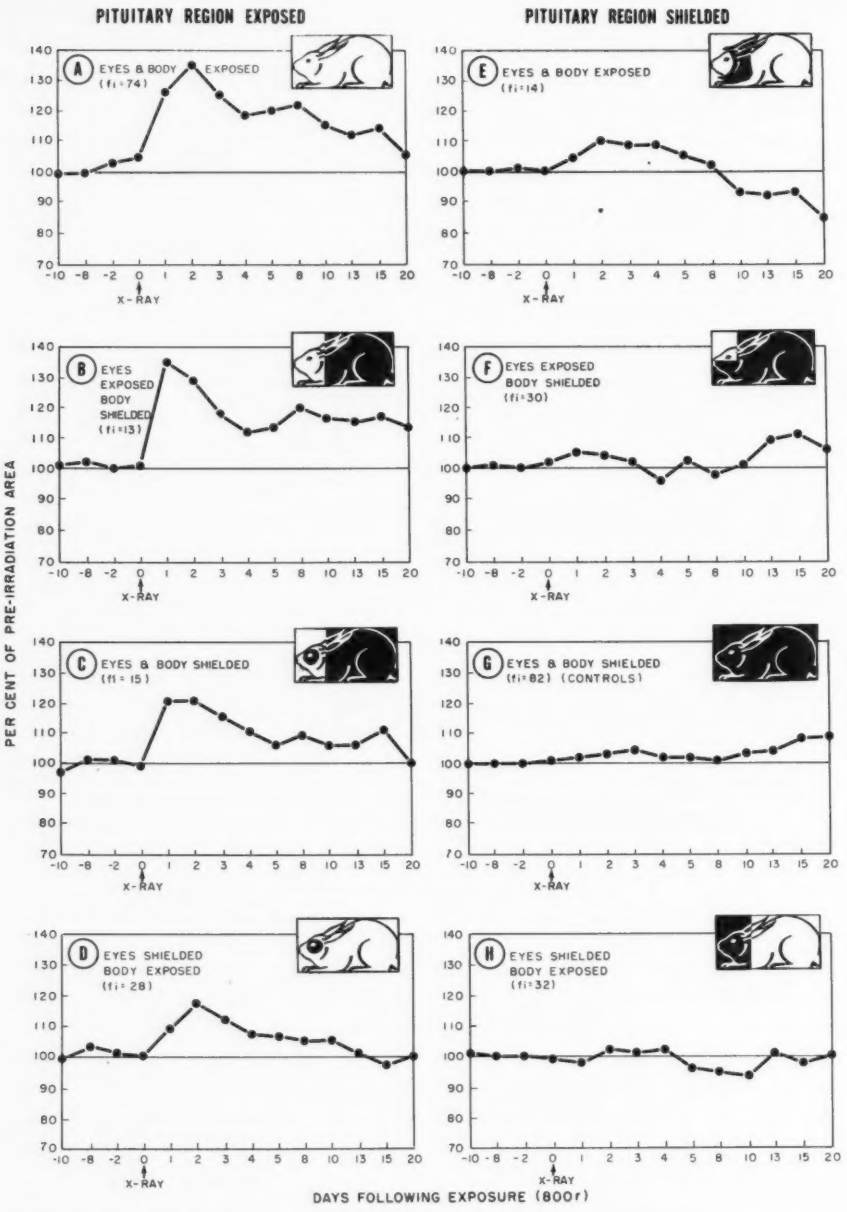
PITUITARY REGION EXPOSED
PITUITARY REGION SHIELDED
A EYES & BODY EXPOSED (fi=74)
E EYES & BODY EXPOSED (fi=14)
B EYES EXPOSED BODY SHIELDED (fi=13)
F EYES EXPOSED BODY SHIELDED (fi=30)
C EYES & BODY SHIELDED (fi=15)
G EYES & BODY SHIELDED (fi=82) (CONTROLS)
D EYES SHIELDED BODY EXPOSED (fi=28)
H EYES SHIELDED BODY EXPOSED (fi=32)
X-RAY
PER CENT OF PRE-IRRADIATION AREA
DAYS FOLLOWING EXPOSURE (800r)

FIG. 3. Changes in area of intraocular endometrial implants in all groups studied. Each point represents the mean area for a given number of functional implants when the average pre-irradiation implant area is expressed as 100%.

3) *Eye:* a lead cup (2.5 in. in diameter) which could be affixed over the eye. See Figure 3D.
4) *Pituitary region:* a lead harness (0.125 in. in thickness) of sufficient area to shield the head region dorsally from the anterior border of the frontal bone of the skull to the external occipital protuberance and which included the cervical vertebrae. Laterally, it covered the area from the posterior margin of the orbital cavity to the superior border of the scapula (see Fig. 3E).
5) *Body (excluding eyes):* as in (1). See Figure 3F.

The sequence of implant growth in animals exposed to 800 r while in the various combinations of shields is summarized in Figure 3. Activation of the endometrial implants occurred only in those situations in which the shielding arrangement permitted exposure of the pituitary region to ionizing radiation (compare A, B, C, and D with E, F, G, and H in Fig. 3). It may be observed that exposing the head and shilding the body (Fig. 3B) did not result in any significant deviation from the pattern of response seen in the totally exposed animals (Fig. 3A), indicating that whole body irradiation was not necessary to obtain the uterine response. Shielding of the target tissue, either alone (Fig. 3D) or together with the body region (Fig. 3C) resulted in a lessening of the response. Thus, it appeared necessary to expose the target tissue as well as the pituitary region to obtain a maximum response. When either the eye region only (Fig. 3F) or the body with the exception of the head region (Fig. 3H) was irradiated the pattern of response of the implants was not altered appreciably from that observed in nonirradiated (equivalent to total shielding) animals (Fig. 3G).

Gross uterine and ovarian response following 800 r total body irradiation: In an effort to determine whether or not the growth response apparent in the endometrial implants following whole body irradiation would be duplicated in the uterus *in situ*, 8 animals were exposed to 800 r total body irradiation and 8 others were sham irradiated. The animals were sacrificed at

TABLE 1. RESPONSE OF PRIMARY AND SECONDARY SEX TISSUE TO 800 r WHOLE BODY IRRADIATION

Time of sacrifice post irradiation	Gross appearance at necropsy		
	Ovaries	Oviducts	Uterus
hours 24 to 36 (2)	Average size: cream colored: some medium-sized V.F.[1]	Slightly hyperemic: becoming edematous	Mature: vascular: slightly motile (typical estrous)
42 to 72 (3)	As above with numerous V.F. on surface: several "mature" V.F.	Hyperemic: edematous: motile	Large: edematous: hyperemic: rhythmically motile: endometrium highly developed (raised into "plush-like" plicae)
92 to 100 (3)[2]	As above plus fresh C.H.[3] on both ovaries	As above	As above

( ) Number of animals.
[1] V.F.—vesicular follicle.
[2] Two of the animals had been exposed to 800 r whole body irradiation 2 months previously. They responded to the second exposure but to a lesser degree (see text under Results)
[3] C.H.—corpus hemorrhagicum (cystic hyperemic follicle).

selected intervals. From the macroscopic changes observed (Table 1) it was apparent that by 42 to 72 hours after irradiation follicular stimulation had occurred. Concomitant with this follicular development was an increasing estrogenization of the secondary sex tissues. Both uteri and oviducts were highly vascular, having the color of arterial blood indicating that they were under the influence of estrogen at this point and not progesterone (Reynolds, 1950). The rhythmical motility of both uteri and fallopian tubes bore further testimony to a rising level of circulating estrogen. Fresh corpora hemorrhagica (or hyperemic cystic follicles) were present in the ovaries of animals sacrificed at 92 through 100 hours post irradiation. Estrogen effects were still visible in the uteri of such animals. Moreover, in 2 animals exposed to whole body irradiation 2 months previously the ovaries displayed an attempt to respond to the second exposure even though they were small, flattened, and almost afollicular. Small, but numerous, fresh corpora hemorrhagica appeared on the surface of both ovaries. The uteri of these animals did not appear as anestrous or castrate, but were more like the uterus observed in a typical estrous rabbit. It appeared that the response of the genital tissues to a second irradiation was delayed and less intense, and that it was probably dependent upon the degree to which the ovarian tissue had recovered from the effects of the initial exposure to ionizing radiation applied some 60 days previously.

The 8 control animals, sacrificed 48 to 168 hours after sham irradiation, did not show any indication of ovarian stimulation or uterine development beyond that usually observed in the mature, non-stimulated rabbit.

DISCUSSION

The effects obtained may have been mediated either through the direct action of x-rays upon the pituitary or indirectly through the action of x-rays upon some neurohumoral regulator in the irradiated pituitary-hypothalamic area acting upon the pituitary. From the present data it is not possible to do other than speculate as to which alternative may be operative. However, the pattern of response noted immediately following x-irradiation in the endometrial implants in those animals in which the hypophysial area was exposed to x-rays is quite similar to that described for intraocular endometrial implants in the adult female rabbit following coitus (Krichesky, 1942). It is now generally accepted that in the female rabbit coitus initiates the release by the hypothalamus of a chemotransmitter into the hypophysial portal vessels (Wislocki and King, 1936; Wislocki, 1937, 1938) which in turn excites the adenohypophysis (Green and Harris, 1947) to discharge gonadotropins. Perhaps radiation may have been instrumental in initiating a similar chain of events culminating in a growth response in the endometrium.

The macroscopic examination of the genital tract of whole body irradiated rabbits, as described in the foregoing results, provides additional

evidence of an increased estrogen level following exposure to ionizing radiation. Also indicative of a rise in circulating estrogen and coinciding with the growth pattern were the vascular changes noted in the endometrial intraocular transplants. The color of the endometrium was most intense when the area was greatest, suggesting a high estrogen level, and was least at the low point in implant size, suggesting estrogen withdrawal. Such vascular changes in endometrial implants have been demonstrated by Markee and French (1934) to parallel the estrogen level in normal female rabbits. The regression seen in the endometrial implants following the initial increase in area after irradiation (Fig. 3A, B, C, and D) parallels that reported by Markee and Anderson (1934) for endometrial implants during the first 6 days after coitus. However, if radiation were capable of inducing ovulation, the intraocular endometrium in such animals should have responded to a rising concentration of progesterone with a second cycle of growth and regression (Krichesky, 1942) which normally occurs during the sixth to the twenty-eighth day after coitus. The absence of a subsequent progestational effect could result from the failure of the pituitary of irradiated animals to release or to secrete the critical amounts of FSH and LH necessary to produce ovulation. Studies are in progress to determine the histological changes occurring in implants following irradiation.

Favorable clinical results have been reported for the use of x-rays in the treatment of certain functional endocrine dyscrasias. In such studies either the pituitary or the ovaries (or both) have been irradiated (for an extensive review of the literature see Freed *et al.*, 1948). In the rabbit, at least, irradiation of the pituitary area was of primary importance, whereas irradiation of the ovary alone was ineffective. It is still a point of contention whether or not these results can be attributed to the stimulatory action of ionizing radiation, yet the simplest explanation of the effects obtained in the present study is that a transient stimulation of the pituitary-hypothalamic-gonadal axis can occur in the rabbit after total body irradiation (800 r) or local irradiation of the pituitary region.

SUMMARY

Intraocular endometrial implants in mature female Dutch rabbits responded to the effects of total body x-irradiation (800 r) by an immediate increase in area within 24 to 48 hours post irradiation. This effect was sustained, but to a lesser degree, for at least 8 days post irradiation. In contrast, the implants in sham irradiated controls exhibited only a slight increase in area during 30 days of observation. When animals were regionally exposed to x-rays by the use of lead shields, only those combinations of shields which permitted exposure of the pituitary region resulted in a sudden surge of implant growth.

It appears that either total body irradiation or local irradiation of the pituitary region can effectively alter the pituitary-gonadal axis in this

species as evidenced by intraocular endometrial growth changes and by the macroscopic appearance of genital tissues *in situ*.

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THE INFLUENCE OF VARIOUS ADRENAL AND GONADAL STEROIDS ON THE ACCUMULATION OF ALKALINE PHOSPHATASE IN THE DUODENUM OF THE SUCKLING MOUSE[1]

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JUST before the nursling mouse is normally weaned, its adrenal glands, apparently acting under the influence of the pituitary, incite a 15 to 20-fold increase in the alkaline phosphatase content of the duodenal epithelium (Moog, 1953). Although this increase ordinarily occurs between the 16th and the 18th day, it can be brought about as much as a week earlier by the administration of a single dose of cortisone acetate. It is striking, however, that the precocious phosphatase synthesis induced by exogenous hormone proceeds at the normal rate to the normal maximum, the process apparently remaining under the control of the reacting tissue.

This finding raised the question whether cortisone acetate acts as an unspecific stimulus, in the way that a variety of unrelated substances may promote specific inductive effects in early embryonic development (*cf.* Holtfreter, 1951), or whether it plays a more precise role, as perhaps by participating in an enzyme-controlled reaction leading to phosphatase synthesis. If the first possibility is correct, one might expect that other steroid hormones and perhaps non-steroid substances as well, would be as effective as cortisone; if the second is correct, only those steroids closely resembling cortisone in molecular structure should be able to activate phosphatase increase. Since no other studies have been made on the control of developmental events by adrenal hormones, there is no evidence bearing directly on this point. It has however been found that desoxycorticosterone acetate is effective in restoring intestinal phosphatase after adrenalectomy in the adult guinea pig (Kutscher u. Wust, 1942) and rat (Verne et Hebert, 1948; Stenram, 1951); in the latter animal Verzar, Sailer, and Richterich (1952) found desoxycorticosterone and cortisone to be equally efficacious, and even gonadal steroids may have an influence (Verne and Hebert, 1948).

The duodenal mucosa of the unweaned mouse passes through a series of phases of increasing sensitivity to cortisone (Moog, 1953). Between 8 and

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14 days a marked and persisting response begins about 48 hours after injection; between 13 and 15 days the response is stronger and faster, presumably because the mucosa, at this time at the threshold of the normal upsurge of phosphatase, has been "conditioned" by endogenous corticoid (Moog, Bennett, and Dean, 1954). In the studies reported in this paper, a variety of adrenal and gonadal steroids has been administered in both of the phases just described. The results show that effective stimulation to phosphatase increase requires a molecular configuration resembling that of cortisone acetate, with the requirements being more strict in the early "unconditioned" than in the later "conditioned" epithelium.

MATERIALS AND METHODS

The steroids used were hydrocortisone (compound F) as acetate and as free alcohol, 11-dehydrocortisone (compound A) acetate, corticosterone (compound B), desoxycorticosterone as acetate and as β-glucoside, Reichstein's substance S, progesterone, testosterone propionate, and estradiol benzoate.[2] Tetrahydrocortisone was also used, but the sample was later found to be contaminated with active material. The effect of cortisone acetate (compound E) was also rechecked. All steroids were made up in saline with suspending agents and benzyl alcohol as preservative, and were injected intraperitoneally. Usually a single injection was made, except that when it was necessary to administer more than 0.05 ml. to animals weighing less than 5 gm., the dose was divided into two portions given 4 hours apart. Doses ranged from 0.15 to 1.0 mg. of steroid per 5 gm. of body weight. With one exception, all hormones were tested on mice at 9–10 days of age, the animals being sacrificed 48 or 72 hours later, and also at 14 days, with sacrifice 48 hours later. Controls, which were always littermates of the treated animals, were injected in the 14-day series with equivalent amounts of saline, but were not injected in the 9–10 day group, since it had previously been observed that injection alone has no effect on intestinal phosphatase at this time.

The mice used belonged to the same colony of white Swiss that provided material for earlier reports (Moog, 1951, 1953). After hormone administration the treated animals and their controls were weighed daily. They were killed by severing the spinal cord, and a segment of duodenum extending 6–8 mm. beyond the entrance of the common bile duct was quickly excised, weighed, and homogenized in iced distilled water. Alkaline phosphatase was then determined on an aliquot of the homogenate by the King-Armstrong technique. The sex of each animal was recorded. Nitrogen was not determined because it had previously been shown that N content does not change much during the period under consideration, and is not influenced by exogenous cortisone (Moog, 1951, 1953).

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RESULTS

A. Injection at 9 or 10 days

1. *The effect on intestinal phosphatase*—The duodenal phosphatase activities of animals treated with hormones at 9 or 10 days, and killed at 11 or 12 days, are summarized in Table 1. Although 2 or 3 members of each litter used were run as controls, all control values have been added together in the table, since the variation at this age is not great (see Fig. 1 in

TABLE 1. THE DUODENAL PHOSPHATASE CONTENT OF 11- OR 12-DAY OLD MICE, FOLLOWING INJECTION OF STEROID HORMONES AT 9 OR 10 DAYS. PHOSPHATASE ACTIVITY IS GIVEN AS GAMMA PHENOL LIBERATED FROM EXCESS PHENYLPHOSPHATE SUBSTRATE BY 1 MG. OF FRESH DUODENAL TISSUE IN A HALF HOUR. THE LAST COLUMN REPRESENTS WEIGHT CHANGE DURING 24 HOURS FOLLOWING INJECTION OF A GIVEN QUANTITY OF HORMONE

Substance	Quantity (per 5 gm. body wt.)	Time	No. of animals	Phosphatase activity			Weight change
				Mean	Range	% difference*	
	mgs.	*hrs.*					*gm.*
None			37	208	156–292		+.49
E acetate	0.15	48	6	1012	840–1220	385	−.23
	0.15	72	4	3184	2790–4110	1431	
F acetate	0.15	48	7	688	574–799	231	−.30
	0.15	72	7	1456	845–2446	600	
	0.50	72	4	3100	3080–3120	1390	−.24
A acetate	0.15	48	7	887	824–997	322	−.08
	0.15	72	10	668	400–1090	221	
Cpd. B	0.15	48	6	543	419–674	163	0
	0.15	72	10	805	353–1400	276	
F alcohol	0.15	48	3	286	255–317	(+27)	+.35
	0.15	72	3	250	246–271	(+23)	
Tetrahydro-cortisone	0.15	48	4	205	189–222	(−1)	
Progesterone	0.30	48	3	200	190–222	(−4)	+.42
	0.30	72	3	192	178–198	(−8)	
	1.0	48	5	1190	1010–1480	476	−.06
DC glucoside	0.15	48	6	232	191–285	(+12)	+.28
	0.15	72	3	233	231–238	(+12)	
	0.50	48	3	241	233–249	(+16)	−.05
	0.50	72	5	229	215–240	(+5)	
	1.0	48	4	726	614–824	250	0
DC acetate	0.50	48	5	218	192–230	(+5)	+.02
Estradiol benzoate	0.15	48	3	238	213–249	(+14)	+.48
	0.15	72	2	249	220–278	(+20)	
	1.0	48	4	231	202–264	(+11)	+.50
Testosterone propionate	0.15	48	2	213	211–215	(+2)	+.65
	0.5	48	3	190	182–208	(−9)	+.60
	0.5	72	3	198	196–201	(−5)	
	1.0	48	4	199	162–236	(−6)	+.61

* % difference = exp. − cont./cont. × 100. Differences in parentheses are not considered significant.

Moog, 1951). The mean value for all controls was 208 gamma of phenol released from excess phenylphosphate substrate by 1 mg. of fresh tissue in a half hour period at 37°C. In normal development the value at the threshold of the period of rapid corticoid-stimulated maturational increase (14–15 days) is about 400 gamma, rising to 4000–6000 gamma by 18 days. In the experiments reported in this paper, an increase that carries the phosphatase content above the 400 gamma level (i.e., at least 100% above our mean 11–12 day value) is considered significant.

The Table shows that only the 11-oxygenated corticosteroids are strongly effective in raising the duodenal phosphatase level in the early period. Hydrocortisone acetate most closely resembles cortisone acetate itself in that it produces an increasing effect up to 72 hours, although the dosage must be raised to obtain a 72-hour effect equal to that of cortisone. Compound A acetate, despite the strong influence it exerts within 48 hours, is not able to sustain the increased phosphatase content produced. Whether compound B, the least effective of the group in this test, induces an effect that continues up to 72 hours is not clear, for the 72-hour values, though averaging higher than those obtained after 48 hours, are extremely variable and include the entire 48-hour range. The ineffectiveness of hydrocortisone as free alcohol, which is much more soluble than the acetate, probably indicates that the substance is metabolized and excreted before it can affect the development of the duodenum. Progesterone and desoxycorticosterone are without influence at low doses, but bring about a substantial elevation of phosphatase content at the high dose of 1 mg. per 5 mg. of body weight; estradiol benzoate and testosterone propionate are not effective even at this level. Tetrahydrocortisone is ineffective at 0.15 mg. per 5 gm.; the results obtained with larger quantities are omitted from the table because the sample was found to be contaminated with active material.

2. *The effect on body weight*—That cortical hormones arrest growth and cause weight loss is well-known. In the young mouse the premature duodenal phosphatase increase elicited by exogenous cortisone is always accompanied by weight loss (Moog, 1953); moreover, during the period from 15 to 17 days, when endogenous corticoids are presumably promoting phosphatase synthesis, the growth rate is retarded (MacDowell, Gates, and MacDowell, 1930; Moog, Bennett, and Dean, 1954). These facts raise the question whether growth arrest is invariably concomitant with, and perhaps contributory to, differentiative activities in the intestine and possibly in other organs.

The last column in Table 1 summarizes the weight changes in all animals during the 24-hour period following injection (in most cases, weight increase is resumed between 24 and 48 hours). With very few exceptions the animals used weighed between 4.0 and 5.5 gms. at the beginning of the experiments. The figures show that a significant elevation of phosphatase is in all cases accompaned by growth failure, although weight loss does not

always occur. The reverse however is not true, since growth may be slowed (0.15 desoxycorticosterone glucoside) or halted (0.5 mg.) without any parallel effect on phosphatase synthesis. Nor is the extent of growth inhibition directly correlated with the extent of enzyme increase: Compound A acetate has a much milder effect on weight than equal amounts of E or F acetate, and a small quantity of compound B, or large quantities of desoxycorticosterone or progesterone, also influence enzyme content more strongly than growth.

B. Injections at 14 days

Table 2 summarizes the results obtained when hormone is administered at 14 days and the animals sacrificed at 16 days. Since the normal varia-

TABLE 2. THE DUODENAL PHOSPHATASE CONTENT OF 16-DAY OLD MICE FOLLOWING INJECTION OF STEROID HORMONES AT 14 DAYS. FOR EXPLANATION, SEE TEXT AND TABLE 1

Substance	Quant., mg.	Phosphatase activity						% difference
		Controls			Experimentals			
		No.	Mean	Range	No.	Mean	Range	
E acetate	0.15	3	650	522–781	5	2202	1892–2355	239
	0.15	3	1600	1250–1890	5	2703	2450–3300	69
F acetate	0.15	3	510	422–618	4	2190	1742–2845	329
	0.15	4	764	594–977	4	2970	2300–4510	292
	0.15	3	1160	1010–1263	5	2573	1650–3634	122
A acetate	0.15	3	700	571–892	4	1780	1590–1932	154
	0.15	5	2424	2110–2637	6	4280	3320–4585	72
Cpd. B	0.15	3	737	702–756	5	1600	1120–1862	118
	0.15	5	2420	2108–2587	6	3540	2470–4540	47
F alcohol	0.15	4	872	643–1470	6	1246	777–1642	(+43)
	0.30	3	1212	1099–1350	3	1514	1390–1714	(+25)
Tetrahydrocortisone	0.30	3	699	510–862	4	722	489–821	(+3)
Progesterone	0.30	3	564	391–718	3	760	490–1034	(+35)
	0.30	3	1770	1311–2033	3	1623	1480–1745	(−3)
	0.50	3	628	552–831	4	822	625–1072	(+20)
	1.0	3	746	523–895	3	1842	1295–2386	149
	1.0	3	1792	1422–1985	3	3620	3069–4591	110
DC glucoside	0.3	5	586	485–770	7	743	504–991	(+27)
	0.5	3	746	523–895	3	871	753–889	(+20)
	1.0	3	726	503–867	4	1920	1810–2028	165
Subs. S	0.3	4	1333	1100–1669	5	1444	1012–2080	(+8)
Estradiol benzoate	0.5	3	893	733–1026	5	1155	980–1342	(+29)
	1.0	3	670	636–690	5	1052	804–1313	57
	1.0	3	1615	1180–1874	7	2544	2024–2930	52
Testosterone propionate	0.3	5	603	510–732	4	709	488–995	(+18)
	0.5	3	1288	1000–1422	5	1428	1080–1763	(+12)
	1.0	—see table 3						

tion at 16 days, which is in the midst of the pre-weaning upsurge of phosphatase content, is very great, results for the treated specimens may be compared only with those in littermate controls. Values obtained from 2 or more litters have been combined in the Table when the control values fell into the same range, but not otherwise. The significance of the differences obtained in this series is more difficult to evaluate, because the percentage differences vary with the absolute level of activity in the controls. Only cases meeting the following criteria are therefore considered to reveal a significant effect: (a) the difference between the experimental and control values is more than 40%; (b) the ranges of experimental and control values overlap only slightly or not at all.

The Table shows that in the sensitive 14-16 day period, hydrocortisone acetate is the most effective compound, somewhat surpassing cortisone acetate. 11-dehydrocorticosterone acetate and corticosterone also have a strong effect which is, however, well below that of the 17-hydroxy corticoids. Progesterone and desoxycorticosterone glucoside are again effective at a high dosage level, and substance S fails to exert any influence at 0.3 mg. per 5 gm. body weight. Hydrocortisone acetate as free alcohol produces very little acceleration, even at 0.3 mg. tetrahydrocortisone is ineffective at the same level.

Extradiol benzoate and testosterone propionate, on the other hand, are more efficacious than in the early period. The former compound at a dosage of 1.0 mg. per 5 gm. exerts a small but significant effect. As is true in all cases already cited, this effect is equal in the two sexes. Testosterone, however, at the high dose level, accelerates phosphatase accumulation slightly in females, but strongly in males (Table 3).

The influence of the injected substances on body weight is less striking

TABLE 3. THE DUODENAL PHOSPHATASE CONTENT IN 16-DAY OLD MICE OF FOUR LITTERS AFTER INJECTION OF 0.1 MG. TESTOSTERONE PROPIONATE AT 14 DAYS. FOR EXPLANATION, SEE TEXT AND TABLE 1

Litter	Controls			Experimentals				% difference
	No.	Mean	Range	No. ♂	No. ♀	Mean	Range	
A	3	1344	1266–1422	2		3085	3080–3090	135
					3	1768	1689–1860	32
B	2	1885	1380–2382	3		4073	3482–4570	117
					3	2473	2220–2836	31
C	3	850	800–875	3		1630	1132–1929	92
					2	1132	1078–1180	33
D	3	1122	1040–1155	2		3076	3000–3152	195
					5	1533	962–1720	37
	Mean % difference, all litters							♂ 135 ♀ 33

than in the 9–10 day series, since the period from 14 to 16 days is a time at which the young normally grow slowly or not at all. The results nevertheless reveal the same general situation as before: precocious phosphatase increase is always accompanied by slight but consistent weight loss, but the parallelism between the two phenomena is not strict.

DISCUSSION

The results just presented demonstrate with some conclusiveness that the acceleration of phosphatase synthesis in the duodenum of the unweaned mouse can be effected only by substances of fairly specific molecular configuration. In the early period, before the intestinal epithelium has been sensitized by endogenous adrenal activity, only the 11-oxygenated corticosteroids can exert a strong influence at a low dose level (Table 1). The 11-desoxycorticosteroids are markedly less effective, and other steroids are inert even at a dose level of 1 mg. per 5 gm. body weight. Evidently then precocious phosphatase increase is most readily brought about by steroid hormones of the type known to intervene in carbohydrate and protein metabolism (*cf.* Dorfman, 1954).

A strict comparison of the effectiveness of the four 11-oxycorticosteroids is not possible, for two reasons. First, the compounds vary in solubility, and since the end result under study is slow, the more soluble materials may be excreted before they are able to exert any lasting effect; cortisone-t for example has been shown to be excreted within 24 hours in adult mice (Bradlow, Dobriner, and Gallagher, 1954). Probably this is the explanation for the ineffectiveness of hydrocortisone as free alcohol, since this quite soluble substance is extremely effective in promoting phosphatase synthesis in chick embryo duodenum in tissue culture (Moog and Nehari, 1954). Second, the liver or some other organ might alter one compound more than another before any changes are brought about in the intestine. Yet these considerations are actually of limited importance, for the substances having the lowest water-solubility are not the most active (e.g., E acetate is slightly soluble in water, compound B practically insoluble); and as the previously mentioned results on explanted tissue show, some corticoids can act directly, without the help or hindrance of any extra-intestinal influences within the organism.

With these considerations in mind, we may then point out that hydrocortisone acetate most closely resembles cortisone acetate in its effect. This is most clearly demonstrated by injection at 14 days (Table 2), but it is true in the early period also, for hydrocortisone acetate is the only other substance that produces a very strong effect that continues for 72 hours (Table 1). Taking into account all the results reported, the order of effectiveness in promoting precocious phosphatase synthesis is as follows: E acetate, F acetate, A acetate, B. It is interesting that in a variety of glyco-

gen-deposition, work, and stress tests, compounds E and F, which are 17-hydroxy compounds, are more effective than compounds A and B, in which the 17-hydroxy group is lacking (Dorfman, 1954).

In the period of high sensitivity, beginning around 14 days, the reaction to the 11-desoxy compounds (desoxycorticosterone, progesterone, substance S) is not much greater than before (table 2). But estradiol benzoate and testosterone propionate now become effective at doses of 1 mg. per 5 gm. body weight. In the case of the female hormone the effect is not great, and is about equal in the two sexes. Testosterone has but a small influence on duodenal phosphatase in females, but in males it becomes as effective as equivalent amounts of desoxycorticosterone or progesterone. Possibly this difference only reflects the ability of the female organism to destroy the male hormone. On the other hand it may be that in the male, gonadal hormones participate in the conditioning of the intestinal mucosa in such a way that by the end of the second postnatal week the mucosa acquires special sensitivity to testosterone (toward all other hormones tested the reactivity in males and females is the same). This view is in accord with Verne and Hebert's (1948) finding that male rats must be castrated as well as adrenalectomized to bring about maximal depletion of intestinal phosphatase. In the adult mouse neither castration nor testosterone administration alter intestinal phosphatase activity (Kochakian and Fox, 1944), but it may be that changes in male sex hormone content can exert an influence only when adrenal stimulation is low or absent.

Whatever the explanation for the testosterone effect may be, it at least seems clear that the hormonal requirements for accumulation of intestinal phosphatase in the nursling are not the same as those for maintaining the enzyme content in the adult. Verzar, Sailer, and Richterich (1952) have shown that desoxycorticosterone acetate is just as effective as cortisone in restoring phosphatase in the intestine of the adrenalectomized rat, and in this laboratory we have found the same to be true in the adult adrenalectomized mouse.

The growth-inhibiting effect of steroids that elevate intestinal phosphatase (Table 1) is particularly interesting in view of the fact that the normal pre-weaning accumulation of the enzyme occurs in a period of negligible growth (MacDowell, Gates and MacDowell, 1930; Moog, Bennett, and Dean, 1954). This correlation between the two events has suggested a casual connection between them: for example, the well-known protein catabolic effect of the corticoids might release a flood of nitrogenous substances utilizable in specific differentiations (*cf.* Engel, 1952; Roberts, 1953). This interpretation may be true in the young mouse, but it must at present be regarded with reserve, for two reasons: (1) The injected young may stop growing because they eat less; this possibility has not been eliminated, although the treated animals are not observed to nurse less frequently than the controls. (2) In the advanced chick embryo, in which the duodenum

is also sensitive to cortisone and hydrocortisone, premature phosphatase elevation occurs in the face of normally rapid growth (Moog and Richardson, in press). In any case, an enhanced supply of nitrogenous substances cannot be the whole explanation for the events described, for hydrocortisone at least exerts a direct effect on explanted tissue (Moog and Nehari, 1954).

Finally it should be emphasized that what has been observed in this study is not a direct activation of phosphatase by corticosteroids; rather, the synthesis of phosphatase is the end result of some other hormone-stimulated process. This fact is particularly clear in the chick embryo, in which corticoids first accelerate the differentiation of the epithelial cells before the enzyme begins to increase (Moog and Richardson, in press). But the delayed response in the young mouse, followed by accumulation at the normal rate to the normal maximum (Moog, 1953), point to the same conclusion. Evidently the accumulation of phosphatase is an aspect of the functional differentiation of the intestinal epithelium that is controlled by a basic metabolic step peculiarly sensitive to corticosteroids of the 11-oxy, 17-hydroxy type.

SUMMARY

The effectiveness of various steroid hormones in elevating the alkaline phosphatase content of the duodenum of the nursling mouse has been determined. The substances used were tested by injection at 9 or 10 days of age (i.e., a week before the maximal level is normally attained), and at 14 days (i.e., at the threshold of the normal upsurge to the maximal level).

At both periods compound F acetate most closely approaches the efficacy of compound E acetate, which was previously studied. The 11-oxygenated corticosteroids which lack the 17-hydroxy group (compound A acetate, compound B) are also effective, but less so than E acetate or F acetate. Dosage of these substances was 0.15 mg. per 5 gm. body weight.

Progesterone and desoxycorticosterone (acetate or glucoside) are effective only at a dose level of 1 mg. per 5 gm. body weight. Substance S is ineffective at 0.3 mg. per 5 gm.

Hydrocortisone as free alcohol has a very weak effect, probably because of its considerable solubility. Tetrahydrocortisone is ineffective.

Estradiol benzoate and testosterone propionate are ineffective up to 1 mg. per 5 gm. body weight when injected at 9 or 10 days. At 14 days the estrogen exerts a weak effect at this dose level; testosterone at the same dosage also exerts a weak effect on females, but is strongly effective in males.

All injections that caused premature phosphatase elevation also resulted in growth inhibition, and frequently in actual weight loss. There was however no strict parallelism between degree of growth inhibition and extent of phosphatase increase.

The results are interpreted to mean that phosphatase synthesis depends on a basic metabolic step that is influenced by corticosteroids of the 11-oxygenated type.

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REDUCTION OF THE NITROGEN-RETAINING EFFECT OF GROWTH HORMONE IN NORMAL AND ADRENALECTOMIZED PANTOTHENIC ACID-DEFICIENT RATS[1]

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INTRODUCTION

CONSIDERABLE data are available indicating that rats subjected to certain nutritional deficiencies are unresponsive to the growth-promoting properties of injected growth hormone. For example, rats given growth hormone while on a vitamin A-deficient diet fail to increase in body weight, have an accelerated onset of deficiency symptoms, and show decreased survival (Margitay-Becht and Wallner, 1937; Ershoff and Deuel, 1945). More recently it has been observed that exogenous growth hormone aggravates the acrodynia of pyridoxine deficiency and fails to produce nitrogen retention in pyridoxine-deficient rats (Beare, Beaton, Smith and McHenry, 1953). Rats deficient in choline (Hall and Bieri, 1953), or in the unsaturated fatty acids (Deuel, Greenberg, Calbert, Savage and Fukui, 1950) failed to increase in body weight when given growth hormone. Gordan, Bennett, Li and Evans (1947) demonstrated that injection of growth hormone in rats maintained on a low protein diet (6% casein) resulted neither in promotion of growth nor in retention of nitrogen; daily supplementation with methionine repaired to a great extent the response to growth hormone. Further studies by Gordan, Bennett, Li and Evans (1948) have shown that the response to growth hormone, as measured by nitrogen retention, is proportional to the dietary protein level when the dietary casein varied from 6 to 24%. In these experiments by Gordan, Bennett, Li and Evans, all animals received the same caloric intake; only the proportion of protein and carbohydrate in the diet varied.

Lotspeich (1950) has shown that "plateaued" female rats on a pantothenic acid-deficient diet do not gain weight when given growth hormone unless the diet is supplemented with pantothenic acid. Recently Beare, Beaton, and McHenry (1954) have reported that rats deprived of panto-

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thenic acid gain less weight when given growth hormone than do animals on the control diet. However, they were unable to demonstrate that growth hormone produced less nitrogen retention in the deficient animals.

It is the purpose of this paper to report data which demonstrate a reduction of the nitrogen-retaining effect of growth hormone in pantothenic acid-deficient rats, and to show that inhibition of this growth hormone activity is not dependent on the adrenal changes which occur in pantothenic acid deficiency.

METHODS

Three experiments were conducted which were designated as Series I, II and III. All animals used were male rats of the Long-Evans strain which, at 30 days of age, were placed on a purified diet[2] deficient in or supplemented with pantothenic acid. Each animal was kept in an individual metabolism cage and fed at the same time each day. Urine samples were collected daily at the time of feeding. Total urinary nitrogen was determined by the micro-Kjeldahl procedure. The control animals were pair-fed, one day after the deficient group, with a diet identical to that of the deficient group, except that the vitamin mixture was complete in all respects. The data presented here include only those animals which showed the typical symptoms characteristic of pantothenic acid deficiency. Greying of the fur, "spectacled" eyes, porphyrin deposition, and diminished hair growth were used as criteria of the deficiency.

RESULTS AND DISCUSSION

Series I: Total urinary nitrogen determinations for 4 deficient and 4 pair-fed control rats were begun after the animals had been given the experimental diets for 57 days. After 6 days of a control preinjection period, during which the average nitrogen excretion did not vary more than 20 mg. from day to day for either group, growth hormone[3] was injected. Each animal received 0.5 mg. of the hormone intraperitoneally per day for the first three days, and 1.0 mg. per day for the 4th and 5th days. The average daily food intake and urinary nitrogen for each group of animals are shown in Figure 1. When 0.5 mg. of hormone was given per day, the average nitrogen excretion of the deficient animals decreased only 33 mg. from the

[2] The pantothenic acid-deficient diet consisted of 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated cottonseed oil (Crisco or Primex), and 4% salts. Crystalline vitamins per kilogram of diet were: 300 μg. D-biotin, 5 mg. 2-methyl-1,4-naphthoquinone, 5 mg. thiamine HCl, 5 mg. pyridoxine HCl, 5.5 mg. pteroylglutamic acid, 10 mg. riboflavin, 10 mg. p-aminobenzoic acid, 20 mg. nicotinic acid, 400 mg. inositol, and 1.0 gm. choline chloride. Each rat received weekly a fat-soluble vitamin mixture containing 800 I.U. vitamin A, 115 chick units vitamin D, 6 mg. synthetic DL-alphatocopherol, and 650 mg. corn oil (Mazola). Control rats received the identical diet supplemented with 50 mg. d-calcium pantothenate per kilogram of diet. We are greatly indebted to Dr. Thomas H. Jukes, Lederle Laboratories, to Dr. Randolph Major of Merck and Company, Inc. and to Dr. Elmer L. Sevringhaus of Hoffman LaRoche, Inc. for generous supplies of crystalline vitamins.

[3] The authors wish to thank Dr. Choh Hao Li for generous supplies of purified growth hormone, Lot L-2333 M.

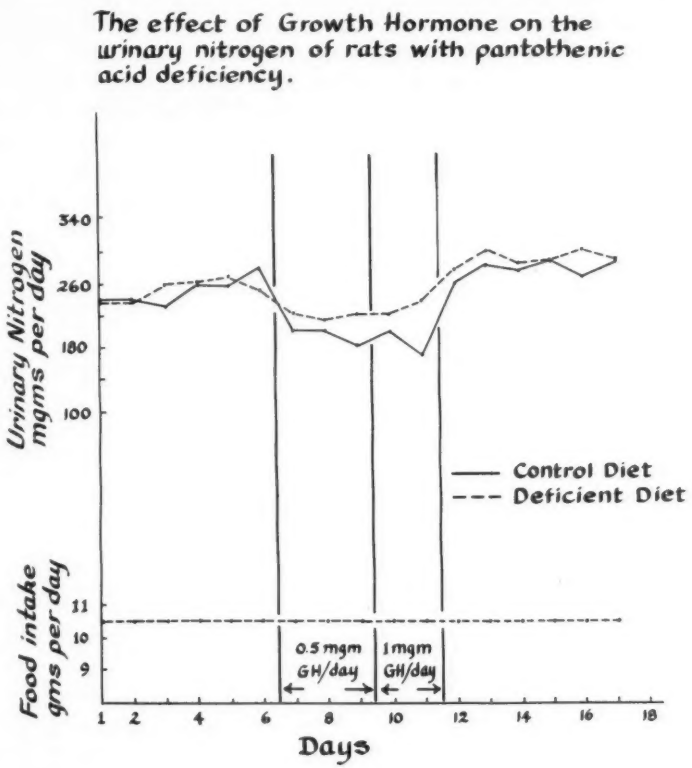

FIG. 1 (Series I) Each curve for urinary nitrogen is the average of 4 rats.

average preinjection control level of 256 mg. Increasing the hormone dosage on the 4th and 5th days caused no further decrease in the amount of nitrogen excreted. The pair-fed control animals, however, showed an average daily decrease of 75 mg. of urinary nitrogen per rat during growth hormone administration. Statistical analysis of the data shows that there was no significant difference in nitrogen excretion between the control and the deficient groups during the preinjection period, but the difference was significant (P-value of 0.01) during the injection period. The daily food consumption was constant for each rat throughout the experiment.

Series II: Ten animals were used in this series, five being placed on the deficient diet, and the remaining five on the pantothenic acid-supplemented diet. After 51 days on the experimental diets, the animals were injected with 0.5 mg. of growth hormone for 3 days, and then with 1.0 mg. for 3 additional days. The data from this series of animals are presented in Figure 2. During the first 3 days when 0.5 mg. growth hormone daily was given, the deficient rats showed an average decrease in nitrogen excretion

of 46 mg. per day, whereas the pair-fed control group decreased approximately 92 mg. per day. When the hormone dosage was doubled, the pantothenic acid-deficient animals did not show a further decrease in urinary nitrogen. In fact, a slight increase in urinary nitrogen occurred during the final two days of the injection period. In contrast, the increased dose of growth hormone caused an increased retention of nitrogen in the control animals. Statistical analysis of the data shows that during the preinjection

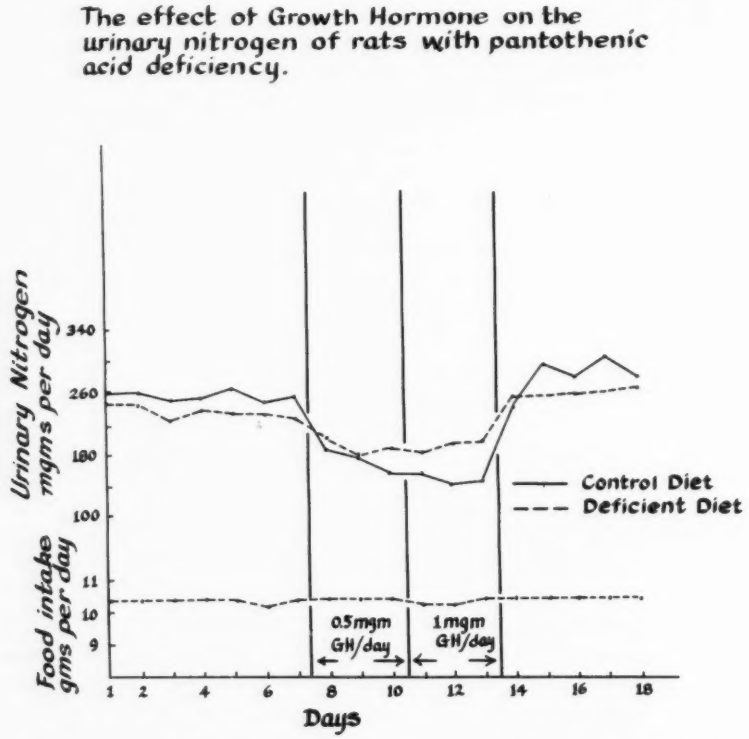

FIG. 2 (Series II) Each curve for urinary nitrogen is the average of 5 rats.

period, there was no significant difference in nitrogen excretion between the deficient and the pair-fed control groups. However, during the injection period, a significant difference in nitrogen excretion occurred, the P-value being less than 0.01. These findings essentially confirm those of Series I.

Series III: Hypertrophy of the adrenals occurs during pantothenic acid deficiency (Hurley and Morgan, 1952), and it has been proposed that there is an hyperfunction of the adrenal with consequent exhaustion and hypofunction. Adrenal hyperfunction might be associated with resistance to

growth hormone. In order to determine whether the adrenal glands were concerned in this observed resistance to growth hormone in pantothenic acid-deficient rats, the animals used in Series II were adrenalectomized, given 2% saline as drinking water, and continued on these respective diets.

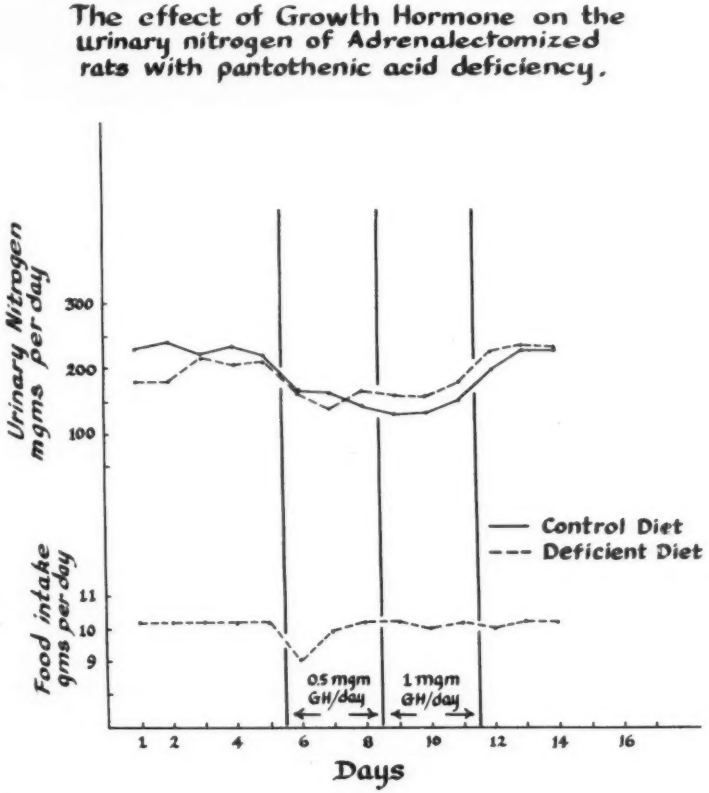

FIG. 3 (Series III) Each curve for urinary nitrogen is the average of 5 rats. These animals were adrenalectomized 7 days prior to starting this experiment.

After a recovery period of 7 days, during which it can be assumed that the circulating cortical hormones had disappeared, daily urine collections were again made, and total urinary nitrogen determined. These animals were given the same doses of growth hormone for the same periods of time as in Series II. With the exception of the first day, the food consumption was constant throughout the injection period.

The data from this series of animals are presented in Figure 3, and the decreased nitrogen-retaining effect of growth hormone shown in the previous experiments is again demonstrated. During growth hormone administration, the average daily urinary nitrogen of the deficient animals

decreased only 40 mg. below the level of the preinjection control period. The urinary nitrogen of the pair-fed controls decreased approximately 85 mg. per rat per day. The difference in urinary nitrogen during the injection period was found to be statistically significant, the P-value being 0.04. As proof that the adrenalectomy was complete, all adrenalectomized rats died within 48 hours when placed on a high potassium diet.

It should be noted that the deficiency produced in these studies was less severe than that usually produced by investigators who have studied metabolic abnormalities in pantothenic-deficient rats, e.g. Winters *et al.* (1952), inasmuch as the rats in the present study were older, 30 days, at the time of starting the deficient diet. Nevertheless, even this mild degree of deficiency interfered markedly with the nitrogen-retaining effect of growth hormone although nitrogen retention was still produced. The greater response to growth hormone injections observed in pair-fed controls receiving the vitamin demonstrated that the reduced response of the deficient animals was due solely to lack of the vitamin and not to a decrease in food intake. Beare *et al.* (1954) had previously suggested that reduction in food consumption could explain the lack of growth promotion by injected growth hormone in riboflavin and in pantothenic acid deficiency. The failure of these investigators to demonstrate a reduction in the nitrogen-retaining effect of growth hormone in pantothenic acid deficiency may have been due to the mildness of the vitamin deficiency produced by starting with rats older than those used in the present study. The possibility of coprophagy with resulting amelioration of B vitamin deficiencies should also be considered when 4 or 5 rats are placed together in the single metabolism cage as in the studies by these investigators.

The findings of Series III indicate that although the adrenal gland may be affected by the vitamin deficiency, it is not responsible for the resistance to the nitrogen-retaining effect of growth hormone. It remains to be shown precisely what mechanism is responsible.

SUMMARY

Male rats of the Long-Evans strain, 30 days of age, were placed on a purified diet deficient in pantothenic acid for 51–57 days. Growth hormone injected at the dose level of 0.5 mg. per rat per day for 3 days, and 1.0 mg. per rat for 2 or 3 additional days decreased the daily urinary nitrogen approximately 40 mg. in two separate experiments. Pair-fed controls receiving the same doses of hormone retained approximately twice as much nitrogen. This resistance to injected growth hormone persisted in the absence of both adrenals.

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BLOOD ACTH IN THE STRESSED ADRENALECTOMIZED RAT AFTER INTRAVENOUS INJECTION OF HYDROCORTISONE[1]

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THE experiments of Ingle *et al.* (1937, 1938), Sayers and Sayers (1945, 1947), Gray and Munson (1951) and Abelson and Baron (1952) have clearly demonstrated that the secretory products of the adrenal cortex inhibit ACTH release in the intact rat. Alterations in the rate of secretion of ACTH were based on changes in weight or the concentration of ascorbic acid of the adrenal glands of the test animal. These indices limit the evaluation of pituitary control since the response in the target gland is measured hours or days after steroid administration. The development of a technic for measuring ACTH in blood has made it possible to study ACTH release in the adrenalectomized animal within a few minutes after administration of hydrocortisone.

METHODS

Male rats from the Holtzman Rolfsmeyer farm weighing 250 to 350 gm. were acclimatized for two weeks in our laboratory (constant lighting and temperature, $78 \pm 1°$ F.).

Experiment 1. Seventy-eight animals were adrenalectomized and given 0.9% sodium chloride solution to drink for two weeks prior to the experiment. During the fourth minute of ether anesthesia, 2 ml. of blood were collected from the aorta of each rat into a heparanized syringe and immediately added to 4 volumes of glacial acetic acid. This blood sample served as a presteroid control. Blood volume was restored by the intravenous infusion of 2 ml. blood from 24- to 48-hour hypophysectomized rats. Following the infusion of blood, 1.0 mg. hydrocortisone[3] was administered intravenously (saphenous vein) during a period of 30 seconds. Exactly 15 seconds after completion of the injection of steroid, 2 ml. blood were withdrawn from the aorta and mixed with 8 ml. glacial acetic acid. This procedure was repeated one minute later. Two ml. hypophysectomized rat

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[3] 2.0 mg. hydrocortisone (in 15% ethanol) per ml.

TABLE 1. BLOOD ACTH AFTER I.V. HYDROCORTISONE

Assay No.	Test substance	Dose per 100 gm. assay rat	Adrenal ascorbic acid depletion (mg./100 gm. adrenal)		Estimate of conc. in mμ per 100 ml. blood M. = S.E.M.
			Individual responses	Avg.	
1	U.S.P. ACTH Std.	1.0 mμ	107, 114, 96, 153, 154, 137	127	
		0.5	157, 48, 94, 101, 65, 91	93	
		0.25	38, 29, 61, −21	27	
	Adrenx rat: bled during 4th minute ether anesthesia	9 ml	60, 154, 102, 106, 100	104	
		4.5	135, 82, 76, 73, 54	84	9.6 ± 1.8
		2.25	40, 31, 56, 57, 0	37	
	Adrenx rat: bled 1¼ minutes after I.V. hydrocortisone	12 ml	67, 116, 203, 99	121	10.5 ± 2.36
		6	81, 101, 139, 75	99	
2	U.S.P. ACTH Std.	1.0 mμ	116, 146, 173, 139, 133	141	
		0.5	77, 111, 30, 104, 103	85	
		0.25	−16, 57, 105, 64	53	
	Adrenx rat: bled 15 sec. after I.V. hydrocortisone	6 ml	89, 98, 107, 64, 113, 69	90	
		3	50, 77, 29, 92, 24, −19	42	8.5 ± 1.8
		1.5	33, 3, 5, −9, 48	16	
	Adrenx rat: bled 4¼ minutes after I.V. hydrocortisone	24 ml.	115, 29, 59, 99, 52, 60	69	
		12	31, 57, 57, 42	47	1.6 ± 0.4
		6	37, −9, −17, 12, −41	−18	
3	U.S.P. ACTH Std.	1.0 mμ	131, 85, 127, 121, 110, 113	115	
		0.5	93, 20, 71, 103, 64, 92	74	
		0.25	45, 81, 53, 17, 61, 29	48	
	50 ml. hypophysectomized rat blood + 4.0 hydrocortisone + 126 mμ ACTH	2.0	115, 151, 128, 216, 172	156	
		1.0	91, 120, 77, 144, 109	108	87.5 ± 17% recovery
		0.5	33, 117, 52, 45, 82	66	

blood were injected by vein after each bleeding. Four minutes later (4¼ minutes after injection of hydrocortisone), the animal was exsanguinated. Seventy-eight rats contributed to each collection period; the blood glacial acid mixtures for each of these collection periods were pooled separately, processed by the oxycellulose[4] technic (Sydnor and Sayers, 1952) and assayed in hypophysectomized rats by the adrenal ascorbic acid-depletion method (Sayers, Sayers and Woodbury, 1948) for ACTH.

Experiment 2. Two ml. of an ethanolic solution containing 4 mg. of hydrocortisone were mixed with 50 ml. hypophysectomized rat blood. The blood-steroid mixture was allowed to stand at room temperature for 1 hour. Two ml. of a 0.01 M HCl solution containing 126 mμ. U.S.P. ACTH standard were then added to the blood-steroid mixture with constant stirring. Fifteen seconds after the addition of ACTH the blood was mixed with 200 ml. glacial acetic acid. This mixture was processed (Sydnor and Sayers, 1952) and assayed in hypophysectomized rats for ACTH (Sayers, Sayers and Woodbury, 1948).

[4] The oxycellulose technic has been modified. Addition of Super-Cel to the diluted blood-acetic acid mixture is unnecessary for the quantitative recovery of ACTH from blood. This step is now omitted.

RESULTS

The results of the two experiments are presented in Table 1 (Exp. 1, assays 1 and 2; Exp. 2, assay 3).

The concentration of ACTH in the control (pre-steroid) blood sample was 9.6 ± 1.8 mμ./100 ml.; that of the samples obtained $\frac{1}{4}$ minute and $1\frac{1}{4}$ minutes after intravenous injection of hydrocortisone was 8.5 ± 1.8 and 10.5 ± 2.4 mμ./100 ml., respectively. The values for the control, the $\frac{1}{4}$ minute, and the $1\frac{1}{4}$ minute samples are in excellent agreement. There was a striking reduction in the concentration of ACTH (1.6 ± 0.4 mμ.) in blood obtained $4\frac{1}{4}$ minutes after steroid injection. The difference between the concentration of the $1\frac{1}{4}$ minute samples is highly significant.

ACTH was quantitatively recovered from blood containing a high concentration of hydrocortisone. The estimate of recovery, $87 \pm 17\%$, is within the limits of error for the oxycellulose technic and bioassay procedure.

DISCUSSION

The blood ACTH titer of the non-stressed rat two weeks after adrenalectomy is 2 to 4 mμ./100 ml. Within 2 minutes after the application of a stressful stimulus the titer has increased to 10 to 15 mμ./100 ml. Neither the imposition of an additional stress nor the prolongation of the stress period for as long as 15 minutes exert an appreciable effect on the high blood ACTH titer providing the blood volume of the animal is not drastically reduced. The titer of ACTH in the blood of the stressed rat with adrenals intact rarely rises above 3 mμ./100 ml. of blood (Sydnor and Sayers, 1954). These and other experiments indicate that two mechanisms are involved in ACTH release: (1) the corticosteroids and (2) a factor other than the corticosteroids which may be the hypothalamus.

Immediately following application of a stress the hypothalamic factor (neurohumor?) may induce a rapid rate of release of ACTH; the increased secretory activity of the adrenal cortex will act to inhibit the adenohypophysis. Subsequently, the titer of ACTH will be determined by the opposing actions of the corticosteroids and the hypothalamic factor.

The sojourn of ACTH in the blood must be taken into account in experiments designed to test the effectiveness of substances in blocking ACTH release. The biological half-life of ACTH in the rat has been demonstrated to be 0.95 to 1.25 minutes (Sydnor and Sayers, 1953). The data presented in this report indicate that ACTH is released at a relatively constant rate for at least $1\frac{1}{4}$ mintues after the intravenous injection of a large dose of hydrocortisone. Between $1\frac{1}{4}$ and $4\frac{1}{4}$ minutes rate of release must be markedly reduced. If one assumes effective blocking was initiated $1\frac{1}{4}$ minutes after hydrocortisone administration it is of interest that the estimate of half-life of the ACTH which exists in the circulation at the initiation of the block turns out to be 1.1 minutes, a figure in excellent agreement with the

values obtained in previous experiments in which the pituitary was removed (Sydnor and Sayers, 1953). It is concluded that hydrocortisone can effectively inhibit ACTH discharge under conditions in which the rate of release is maximally accelerated, and this inhibition can become manifest within a period of 1 to 2 minutes after injection of the steroid. The inhibitory effect, once initiated, apparently goes rapidly to complete block.

Two additional lines of evidence support the conclusion that the reduced blood ACTH titer $4\frac{1}{4}$ minutes after hydrocortisone is due to inhibition of ACTH discharge: (1) Previous experiments demonstrated that the blood ACTH titer of ether-anesthetized rats two weeks after adrenalectomy was 15.3 ± 3.3 at the 3rd, 16.5 ± 4.4 at the 7th, 33.9 ± 9 at the 11th and 53.5 ± 25.6 mμ./100 ml. at the 15th minute of anesthesia. If the blood volume were restored after each bleeding, the titer was 12.1 ± 1.5 mμ. at the 3rd, 10.7 ± 1.5 mμ at the 7th, 15.8 ± 1.8 mμ. at the 11th and 11 ± 1.8 mμ./100 ml. at the 16th minute. In no instance did the blood ACTH titer *decrease* significantly with time after initiation of the stress (Sydnor and Sayers, in press). (2) ACTH can be recovered quantitatively from blood containing a high concentration of hydrocortisone.

Gray and Munson (1951) have concluded that the pituitary of the intact rat responds to the inhibitory action of injected corticosteroids within 2 seconds after their injection. The experiments of this report suggest that the pituitary of the stressed adrenalectomized rat does not respond with substantial decrease in ACTH output until 2 minutes after hormone administration. Regardless of these differences in detail, sufficient data have accumulated to indicate that the pituitary responds rapidly to stressful stimuli (accelerated discharge of ACTH) and to corticosteroids (inhibition of release, or block after accelerated release has occurred). The dynamics of ACTH release have been characterized sufficiently to indicate that little or no inertia exists in this component of the pituitary-adrenal system of the rat.

The dose of hydrocortisone employed, namely, 1.0 mg., is relatively large. For that reason the physiological significance of the present experiments is justifiably in question and the study should be extended to include smaller doses.

The mechanism(s) by which adrenocortical hormone(s) influence pituitary ACTH has not been elucidated. According to Sayers and Sayers (1947), it may act *directly* on the adenohypophysis or the hypothalamus, or *indirectly* through some metabolite as a consequence of excess hormone. The short time-lag (less than 2 minutes) required for the inhibitory effect to become manifest is compatible with the concept that the hormone acts *directly* on the adenohypophysis or on the hypothalamus to inhibit the discharge of a neurohumor which effects ACTH release. It does not eliminate the possibility that the effect may be mediated by a metabolite. Definitive proof for hypothalamic control of ACTH must await the isolation from the

hypothalamus of a neurohumor which specifically accelerates ACTH release. Once the neurohumor is isolated it may be possible to establish the locus of action of adrenocortical hormone.

SUMMARY

The concentration of ACTH in blood from ether-anesthetized adrenalectomized rats was determined prior to, and $\frac{1}{4}$, $1\frac{1}{4}$, and $4\frac{1}{4}$ minutes after intravenous injection of 1.0 mg. hydrocortisone/100 gm. rat. The blood ACTH titer of the pre-steroid (control), the $\frac{1}{4}$ minute and the $1\frac{1}{4}$ minute samples were 9.6 ± 1.8, 8.5 ± 1.8, and 10.5 ± 2.4 mμ./100 ml.; the titer of the $4\frac{1}{4}$ minute sample was 1.6 ± 0.4 mμ./100 ml.

In vitro studies indicate that the oxycellulose technic quantitatively recovers ACTH from blood containing 0.08 mg. hydrocortisone/ml. It is concluded that (1) intravenously administered hydrocortisone can effectively block ACTH release in the stressed adrenalectomized rat within 1 to 2 minutes, and (2) a high concentration of hydrocortisone in blood will not interfere with the recovery of ACTH.

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THE EFFECTS OF HYPOPHYSECTOMY AND OF THYROXINE ON THE RADIATION-INDUCED CHANGES IN THE RAT THYROID[1]

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CELLULAR hypertrophy and nuclear changes are impressive histologic features of the thyroid of the rat previously irradiated by the administration of I^{131} (Maloof, *et al.*, 1952). These findings have been observed as early as one month and have persisted for as long as 18 months after a total radiation dose of only 5800 roentgen equivalents physical (5 μc of I^{131}) in spite of an abundant supply of iodine (3 μg./gm. food) in the diet. Not all the cells are equally affected; the abnormal nuclei are scattered, but the cellular hypertrophy is constant and intense. These changes vary, depending upon the initial dose of I^{131}.

It has not been clear whether the cellular hypertrophy and nuclear changes are direct results of the ionizing radiation upon the cells themselves, or whether the cells are conditioned by the radiation, the subsequent changes arising secondarily from increased thyrotropic hormone stimulation resulting from a reduced rate of hormone synthesis by the irradiated thyroid gland. The present study demonstrates that the increase in cell height is prevented by hypophysectomy or the administration of DL-thyroxine after I^{131} irradiation; the nuclear changes are not completely inhibited, but appear less obvious.

MATERIALS AND METHODS

Eighty 125 ± 10 gm. male rats of the Sprague-Dawley strain were individually marked for future identification; two animals were placed in each of forty cages. All animals were placed on a modified low iodine (Remington) diet (0.1 μg. iodine/gm. food) for 14 days; then 40 animals were given 50 μc of I^{131} intraperitoneally (group R), the others were used as controls (group C). The 48-hour retention of radioactive iodide by the thyroids of the animals ranged from 40 to 55%. After the 48-hour retention had been determined, both groups of animals were placed on Purina Fox Chow diet (iodine content = 1 μg./gm. food[2]) until the termination of the experiment.

Twenty-eight days after the I^{131} was given, 12 animals from group R were hypophysec-

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[1] Aided by Grant AT(30-1) 667, from the Atomic Energy Commission.

[2] The iodine content of the Purina Fox Chow has been found to vary from 1 to 3 μg. per gram of food; in this experiment the diet was analyzed on two occasions for its iodine content.

tomized by the parapharyngeal route[3] (R-H) and 12 others from group R were started on a daily subcutaneous injection of 15 μg. of DL-thyroxine[4]/100 gm. of body weight (R-T). Twelve animals from group C were also hypophysectomized as controls (C-H).

Seven and one-half months after irradiation, 6 animals each from groups C, C-H, R, R-H, and R-T were given 3 μc. of I^{131} intraperitoneally and the 24 and 48 hour retention of I^{131} was determined by placing the thyroid area of the animal in close proximity to a 2.2 cm. aperture of a shielded scintillation detector with a sensitivity of 2200 net counts/μc I^{131}.

Eight months after irradiation, 6 animals each from groups C, C-H, R, R-H, and R-T were killed by ether and exsanguination. Thyroxine injections were stopped after 8 months in 6 other animals of group R-T and 2 months later these animals were killed (R-T°). The serum concentration of protein-bound iodine (PBI) was determined in duplicate on 1–2 ml. samples of serum by a modification of Chaney's (1940) method. Body weights were recorded. The organs were weighed on a torsion balance to 0.1 mg. and immediately placed in fixative, the thyroid in Stieve's solution and the pituitary in $HgCl_2$:HCHO (9:1). The thyroid was sectioned at 6μ at several levels and the tissue was stained with Feulgen's reagent and hematoxylin-eosin.

The sella turcica was examined under a dissecting microscope to determine the completeness of hypophysectomy. One animal was excluded because the procedure was incomplete.

RESULTS

There was a 2–3 gm. per day increase in body weight while the rats were on a low iodine diet and a similar increase in all groups in the subsequent

TABLE 1. DATA ON RATS GIVEN I^{131} AND THEN EITHER HYPOPHYSECTOMIZED OR GIVEN DL-THYROXINE

Groups	Body wt. (gm.)	Retention of I^{131} by thyroid		Thyroid wt. mg./100 gm.	Adrenal wt. mg./100 gm.	PBI (γ %)
		24°	48°			
C (Control)	420±13*	20%	15%	5.8±0.9*	12.8±2.7*	2.5±0.5*
C-H (Hypophysectomy)	215±20	7%†	3%	3.7±0.4	4.3±1.0	—
R (50 μc. I^{131})	487±25	21%	13%	5.9±0.5	11.4±1.5	2.7±0.7
R-H (50 μc. I^{131}+hypophysectomy)	196±14	9%†	4%	3.9±0.5	3.8±0.9	0.9±0.6
R-T (50 μc. I^{131}+DL-thyroxine)	406±27	10%	7%	4.7±1.5	12.3±1.8	3.1±0.4
R-T° (50 μc. I^{131}+DL-thyroxine ×8 mos. and no DL-thyroxine ×2 mos.)	450±22	—	—	4.5±0.9	12.7±2.1	—

* Mean of 6 animals and the standard deviation.
† The retention of I^{131} by groups C-H and R-H appear high, however, this may be a technical matter since the retention in the control group (C) is higher than is usually noted in our control groups by a factor of 2.

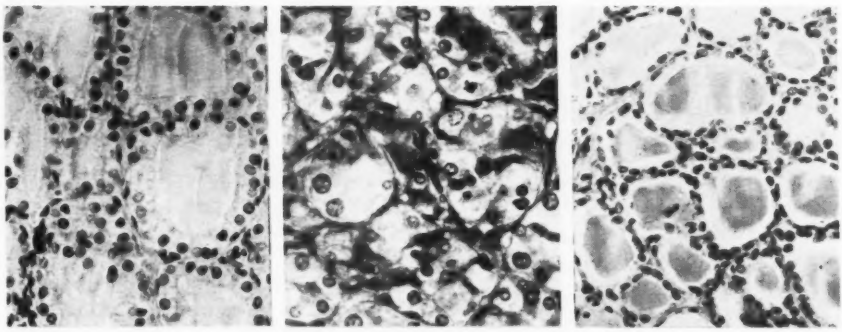
month after the administration of the I^{131} while the animals were on the Purina Fox Chow diet. Thereafter, the control animals (C), the irradiated animals (R) and those on DL-thyroxine (R-T) continued to gain; however, the hypophysectomized groups (C-H and R-H) merely maintained their pre-hypophysectomy weight. Body weights, thyroid and adrenal weights,

[3] For which we are indebted to Mr. L. Nay. These animals were maintained on 5% dextrose as drinking fluid and the Purina Fox Chow diet.

[4] Roche-Organon preparation. Aqueous solution in ampoules of 1 ml. (1 mg. of DL-thyroxine per 1 ml.)

the retention of I^{131} by the thyroid and the PBI are recorded in Table 1. The thyroid weights of both hypophysectomized groups (C-H and R-H) were the same, but were significantly lower than those of the control group (C) and the irradiated group (R) [$P < 0.01$]. However, the thyroid weights of the irradiated animals given DL-thyroxine (R-T) were not statistically different from those of any other group. The weights of the adrenals of the various groups were the same except for the low values in the hypophysectomy groups.

The 48-hour retentions of I^{131} of the hypophysectomy groups were similar, but were significantly lower than in the control group (C) and the irradiated group (R). The administration of DL-thyroxine to the irradiated animals (R-T) similarly depressed the retention of I^{131} but to a lesser degree, probably because of the dose of thyroxine administered. The PBI



A B C

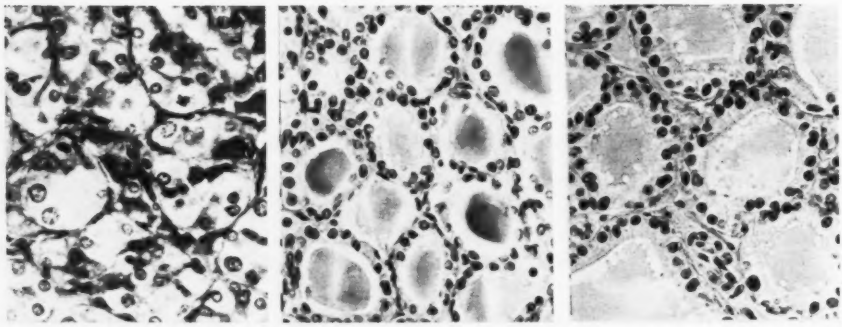
FIG. 1. (A) Normal thyroid of a rat. ×520. (B) The thyroid of a rat given 50 μc. of I^{131} (R) 8 months previously. Note the cellular hypertrophy and the abnormal nuclei. ×520. (C) The thyroid of a rat given 50 μc. of I^{131} 8 months previously and hypophysectomized 1 month after the administration of the isotope (R-H). Note the absence of the histologic abnormalities. ×520.

values were the same in all groups except for the low values in the hypophysectomy group (R-H).

Hypophysectomy resulted in a failure of the cell height to increase in the follicles of the previously radiated thyroid gland. This is illustratd in Figure 1. A normal thyroid from group C (control) is illustrated in Figure 1A; hyperplasia, usually seen in the thyroid of the rat, and the uniform nuclear size are apparent. The marked increase in the height of the follicular epithelium and the nuclear changes which occur after radiation are seen in Figure 1B. The cellular hypertrophy was uniformly noted throughout the thyroids of all animals in this group. The nuclei were large, irregular in outline and hyperchromatic and were found in a majority of the cells in most follicles; however, it was not unusual to discover that

only a few nuclei appeared abnormal in any one follicle. Some nuclei were pyknotic; mitoses were rarely seen. The photomicrograph in Figure 1C is from the I^{131}-hypophysectomy group (R-H); absence of the cellular hypertrophy and of the nuclear changes is evident. Careful examination of the histologic sections, however, disclosed that an occasional abnormal nuclei was present (e.g., follicle in upper left hand corner).

The administration of DL-thyroxine also prevented an increase in the follicular cell height of the irradiated thyroid (Fig. 2). The photograph in Figure 2B (I^{131} and DL-thyroxine) is to be contrasted with that in Figure



A B C

FIG. 2. (A) Same as Figure 1(B). ×520. (B) Thyroid of a rat given 50 μc. of I^{131} 8 months previously and then given DL-thyroxine for 7 months prior to killing (R-T). The absence of the histologic abnormalities is to be noted; however, these changes are not as marked as in Figure 1 (C). ×520. (C) Thyroid of a rat given 50 μc. of I^{131} 10 months previously; daily injections of DL-thyroxine were initiated 1 month after the administration of the isotope and were stopped 2 months prior to killing. ×520.

2A (I^{131}). Absence of the increase in cell height and the nuclear abnormalities is again noted. The microscopic appearance of tissue sections from the thyroxine treated animals (Fig. 2B) was similar to that of sections from the hypophysectomized group (Fig. 1C) but the changes were not quite so marked. The cell height of the follicular epithelium in the thyroxine treated animals (R-T) is higher than in the hypophysectomized group (R-H), but it is still less than in the control animals (C). Abnormal nuclei were more obvious in the thyroxine treated animals than in the hypophysectomized group, yet they were encountered only occasionally in any one thyroid. The difference in the histologic picture between groups R-T and R-H may be related to two factors: (1) the dose of thyroxine[5] administered to group R-T or (2) the removal by hypophysectomy (R-H) of other pituitary hor-

[5] The solution in an occasional ampoule of the DL-thyroxine was slightly discolored and yellow which suggested some decomposition. The actual dose of thyroxine given may, therefore, have been lower than the stated dose. This is suggested by the PBI value and the continued weight gain of group R-T.

mones which singly or synergistically influence cellular growth. The thyroids in group R-T and group R-H were able to concentrate I^{131} as measured by autoradiographs in spite of the depression of thyroid activity.

The cessation of DL-thyroxine (R-T°) permitted the suppressed histologic abnormalities to become manifest (Fig. 2C). There was an increase in the height of the follicular epithelium (*cf.* Fig. 2B) and in the large hyperchromatic nuclei which were present in most follicles.

DISCUSSION

Previous observations from this laboratory suggested an impairment in cell division in the thyroid after I^{131} irradiation (Maloof *et al.*, 1952). Since the irradiated thyroids also had a reduced functional capacity, it seemed that the observed increase in cell height resulted in part from pituitary stimulation probably caused by a deficiency in the production of thyroid hormone.[6] The present study sustains this concept by demonstrating that after I^{131} irradiation, the cell height does not increase when stimulation from the pituitary is either removed by hypophyectomy or inhibited by the administration of DL-thyroxine, but does increase two months after the inhibition has been removed. Hypophysectomy and the administration of DL-thyroxine may also have caused a regression of the cellular hypertrophy that is known to be present one month after the administration of I^{131}.

Although the effect of I^{131} irradiation of the thyroid cell is a complex process, the increase in cell height is probably secondary to alterations in pituitary activity resulting from an induced hormonal imbalance; the nuclear changes are not completely governed by pituitary activity and appear to be a direct effect of radiation. However, by decreasing the stimulation from the pituitary (thyroid stimulating hormone) and thus placing the thyroid in a state of relative inactivity wherein the cells are dividing slowly, nuclear abnormalities are made less obvious. These nuclear alterations and the secondary epithelial hypertrophy are interesting; their implication to radiation carcinogenesis is indefinable at present, but there may be merit in the suggestion of Doniach (1953) that thyroid be administered to patients who have been treated with I^{131} for hyperthyroidism.

As a result of observations on the effects of x-radiation in mice, Kaplan (1949) stated "the induction of lymphoid tumors is not solely the result of a simple direct action of radiation upon the susceptible cell or tissue of origin." In a subsequent work Kaplan and Brown (1952) have provided further evidence as to the nature of the indirect mechanism involved in radiation changes. Recently in discussing the question of carcinogenesis produced by radiation, Kaplan (1954) defined this type of carcinogenesis

[6] Recently Kirkland (1954) has presented evidence which demonstrates that there is impairment in the organic binding of iodine in the thyroid of humans early after I^{131} treatment.

as "an induced imbalance between the growth capacity of tissue and the growth stimulus of tissue." In spite of the fact that Gorbman has intimated that the pituitary tumors appearing in mice after large doses of I^{131} are direct effects of radiation (Gorbman, 1949, 1950, 1952), Goldberg and Chaikoff (1951) have demonstrated that these are secondary effects and can be prevented by the administration of thyroxine after radiation. Recently, Edelmann and Gorbman (1954) have shown that in mice pituitary tumors, which were conditioned by a radio-thyroidectomy dose of I^{131} (30 microcuries), were actually produced by a subsequent non-specific dose of radiation as x-irradiation to the hind limb or the administration of radioactive sodium to the same animal.

It is evident that radiation produces alterations in the metabolism of the thyroid and subsequent morphologic changes which vary depending upon the initial dose of I^{131} (Maloof *et al.*, 1952). The appearance of these abnormalities is modified by the rate of cellular growth and division ("growth stimulus of the thyroid").

CONCLUSION

1. The thyroids of rats which had previously been given I^{131} show cellular hypertrophy and morphologic alterations in the nuclei.

2. The cellular hypertrophy is completely and the nuclear changes are partially inhibited by hypophysectomy or the administration of DL-thyroxine. These latent histologic abnormalities become manifest two months after the cessation of DL-thyroxine.

3. Some of the morphologic changes in the thyroid of the rat after sublethal thyroidal doses of I^{131} appear to be secondary and not primary effects of radiation.

Acknowledgment

The author is indebted to Dr. J. B. Stanburg for helpful suggestions, and to Miss Astra Birins, Miss Betty Rapp, Mrs. Frances Ashodian and Miss Vivian Mays for technical help.

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NOTES AND COMMENTS

FAILURE OF AN ANTIHISTAMINE DRUG TO PREVENT PREGNANCY IN THE MOUSE

Shelesnyak (1952) has reported that an antihistamine drug (Diphenhydramine) inhibits deciduoma formation in the pseudopregnant rat uterus stimulated by histamine or other agencies, and has suggested that histamine may be the physiological stimulus for nidation. These observations raised the question whether an antihistamine drug would prevent implantation in an intact animal.

Swiss white mice weighing about 20 grams were used. The females had successfully given birth to a first litter several weeks previously, but lactation had been terminated by prompt removal of the newborn mice. The males had demonstrated their reproductive competence in previous matings. Females were randomly assigned to drug and control groups (8 to each group). In each cage were placed 2 females and 2 males for a period of 7 days.[1] The females received Pyranisamine[2] maleate, 50 mg./kg. in 0.9% NaCl, or saline solution alone, subcutaneously in 0.5 ml. volume every day at 4 P.M., beginning 3 days before introduction of the males, and terminating after 10 injections, when the males were removed. The females were then isolated in individual cages.

The dose of Pyranisamine was chosen after preliminary experiments had shown that 200 mg./kg. intraperitoneally or subcutaneously caused ataxia and convulsions leading to death in nearly all animals, and that extreme lethargy was produced by a dose of 100 mg./kg. At 50 mg./kg. a pronounced hypnotic action was evident, and obvious reduction of motor activity persisted for about 24 hours. We therefore considered this (*ca.* 1 mg.

TABLE 1. FAILURE OF PYRANISAMINE MALEATE TO PREVENT PREGNANCY IN THE MOUSE

50 mg./kg., s.c., daily to females for 3 days before, and for 7 days during the presence of males

	Pregnant/Total	Litter sizes
Control group	8/8	4, 6, 6, 6, 7, 7, 9, 9.
Drug group	7/8	7, 7, 8, 8, 8, 9, 11.

per mouse) to be the maximal tolerated dose for our purpose. The well-known indifference of mice to injected histamine made it impractical to ascertain whether the adopted dose of Pyranisamine actually had any antihistamine action. As much as 1000–2000 mg./kg. of histamine phosphate failed to kill control mice; indeed, such doses caused only central effects (ataxia, respiratory depression, clonic convulsive activity) whose antagonism (or lack of it) by the antihistamine drug would have been quite irrelevant to the hypo-

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[1] As the duration of the estrous cycle in the mouse is 4–5 days, the 7-day mating period was adopted to avoid the necessity of determining individual ovulation times by vaginal smears.

[2] "Neoantergan."

thetical uterine action of histamine under investigation. It was therefore assumed, by analogy with other species, that Pyranisamine, in dosage sufficient to cause marked hypnotic effects, would be present in high enough concentration to inhibit any of the common smooth muscle or vascular actions of histamine.

The results of the matings are shown in Table 1. All mice in the control group bore litters, and all but one in the drug group did so.[3] There was no significant difference in litter size. All litters appeared grossly normal and were successfully suckled and weaned. Two litters were born on the 20th day after introduction of the males, 11 on the 23rd day, 1 on the 24th day, and 1 on the 25th day. All 7 pregnant females in the drug-treated group bore their litters by the 23rd day. As the period of gestation in the mouse is 19–21 days, and implantation occurs on the 5th day (Snell, 1941), these figures show that ovulation, fertilization, and implantation occurred during the period of drug administration. As Pyranisamine was administered every afternoon and still showed sedative effects 24 hours later, it may be assumed that effective drug levels were present throughout this period.

While one cannot argue from one species to another, it may be questioned whether the very high local concentrations of antihistamine drug employed by Shelesnyak may have inhibited deciduoma formation in the rat uterus by a non-specific mechanism. Controls with substances devoid of antihistamine action might have elucidated this question. The mouse resembles the rat in its unusual indifference to histamine. In the mouse, at least, the present investigation has shown that ovulation, fertilization, and implantation occur normally despite very high dosage of one of the most potent (Bovet and Bovet-Nitti, 1948) antihistamine drugs.

SUMMARY

The antihistamine drug, Pyranisamine maleate, administered daily by subcutaneous injection to female mice, in dosage sufficient to cause marked and prolonged sedative effects, does not interfere with normal ovulation, fertilization, or implantation.

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[3] The single infertile female had borne her first litter only 8 days prior to the start of the experiment, whereas at least 15 days have elapsed for the other females.

PREPARATION OF HIGHLY ACTIVE FOLLICLE STIMULATING HORMONE FROM SWINE PITUITARIES

Recently, Steelman *et al.* (1953) showed that follicle stimulating hormone (FSH) with good biological activity may be prepared from tissue residues resulting from the acid acetone extraction of swine pituitaries. These FSH preparations compared favorably

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with those of Li *et al.* (1949) and Van Dyke *et al.* (1950). Further attempts to purify the swine FSH by classical alcohol and salt methods have been largely unfruitful. However, if the purified FSH prepared by the method of Steelman *et al.* (1953) is digested at 37° C. for three hours with pancreatin (1:90), at an enzyme-substrate ratio of approximately 1:100 there is no loss in biological activity and further purification is possible. Under these conditions the increase in the trichloroacetic acid precipitable nitrogen is 30–40%. Fractionation of the digestion mixture successively with alkaline and acid ethanol at low temperatures has led to the isolation of FSH which is 10 to 11 times as active as the Armour Standard (PF-264-151-X). In the table below are listed the potencies of various FSH preparations in terms of the Armour Standard. Electrophoretic analysis of one preparation with an activity 7 to 8 times standard showed the presence of 5 components the largest of which constituted 35% of the protein present.

In view of our findings, we are led to the conclusion that the swine and sheep FSH preparations now available are impure although physical measurements of some may show a high degree of homogeneity. The possibility exists that there is a difference between the activity of swine and sheep FSH in the pure state but this has not been demonstrated in the case of other pituitary hormones. Additional details of the fractionation procedure will be published at a later date.

Highly active FSH prepared by the above method has been administered to humans by Maddock (1954) and shown to possess good biological activity at low dosage levels. It also stimulates the formation of anti-hormones when given daily for periods longer than 6–8 weeks.

BIOLOGICAL ACTIVITY OF VARIOUS FSH PREPARATIONS

Preparation	Source	Activity (% Armour Standard)*	Reference
Li	Sheep	135 ± 16	Li *et al.* (1949)
Li (11631)	Sheep	93 ± 10	
Van Dyke (446-CC)	Swine	61 ± 9	Van Dyke *et al.* (1950)
PF-593-19B†	Swine	950 ± 160	
PF-593-25B†	Swine	1130 ± 220	

* Assayed by the method of Steelman and Pohley (1953).
† Prepared by the method described above.

SUMMARY

Highly active FSH was prepared by fractionation with ethanol following digestion with pancreatin. Preparations were more active than previously reported yet electrophoretic analysis indicated multiple components.

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GAMMA COUNTING OF RADIOSODIUM AND RADIOPOTASSIUM IN THE BIOASSAY OF ALDOSTERONE AND RELATED STEROIDS[1]

The use of the urinary Na^{24}/K^{42} ratio as an index for the bioassay of adrenal steroids effective in mineral metabolism was first reported by Simpson and Tait (1950, 1952). Tait and Williams (1952) determined the beta ray emanations of radioactive sodium and potassium and discriminated between these elements physically by using differential filtration. We wish to report a simpler and more versatile method of determining radioactive sodium and potassium in biologic fluids by gamma counting of these two elements simultaneously.

Simultaneous counting of radioactive sodium and potassium was accomplished by utilizing the energy response characteristics of the scintillation counter (Hall, 1954; Hofstadter and McIntyre, 1950). The simplicity of this type of counting system permits large numbers of samples to be measured with a minimum of time, since the tedious and exacting procedures for planchette preparation required by beta counting are eliminated. The versatility of gamma counting is that it can be readily adapted to *in vivo* problems involving radiosodium and radiopotassium or other combinations of gamma emitters.

A. THE PHYSICAL SYSTEM

The apparatus consists of a NaI (Tl) crystal optically coupled to a Dumont type 6292 photomultiplier tube. Operating voltage is supplied by dry batteries of 90 volts each connected across the individual dynodes. Pulses from the photomultiplier pass through a conventional cathode follower to a linear amplifier. Two pulse height analyzers with their inputs connected in parallel to the output of the linear amplifier select pulses and pass them to separate scalers. Counts coming from the pulse height analyzer which is set at the low-energy end of the spectrum represent a mixture of Na^{24} and K^{42}, whereas the pulses of higher energy which are passed by the second analyzer are primarily from the 2.78 mev gamma of Na^{24}. Following the notation of Tait and Williams (1952), let N_1 and N_2 represent the counts from the low and high energy channels, respectively. I_1 is the contribution to N_1 of Na^{24} and I_2 is the contribution to N_1 of K^{42}. The channel ratio is defined as $r = N_2/N_1$. For Na^{24} taken alone $r = m$ whereas K^{42} taken alone has $r = n$. The physical ratio

$$S = \frac{m - r}{r - n} = \frac{I_2}{I_1}$$

may be obtained quickly once the channel ratio of the unknown mixture is measured by referring to a calibration graph of S versus r for the particular values of m and n which are essentially defined by the pulse height analyzers.

Certain special problems arise with respect to this application of the scintillation spectrometer. Fluctuations in the values of m and n will occur for all variations of the over-all gain of the system. Good line voltage stabilization and reasonably constant room temperature are necessary to eliminate short-term fluctuations. Long-term drift may be checked with a standard Co^{60} source, if the analyzers are set to count only the upper portion of the 1.31 mev photopeak, since small changes in the circuit parameters will introduce large changes in the Co^{60} count rate at such a setting. A source of variation of m and n at high full spectrum count rates is the coincidence summing of pulse energies (Orvis and Williams, 1954). This effect was insignificant in the present case since all

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[1] The radioisotopes utilized in the studies reported in this paper were supplied by the United States Atomic Energy Commission.

count rates were less than 200 c/s. Effects of sample volume and geometry on m and n were eliminated by diluting all samples to the same volume and by maintaining constant source-counter geometry. Constant geometry counting was also necessary to eliminate the effect of combination peaks (Jastram, Whalen and Zinke, 1952.)

In addition to the factors influencing the final error which have been discussed by Tait and Williams (1952), it is necessary to choose the pulse height analyzer settings so that m and n will be stabilized against small and inescapable fluctuations in the overall amplification of the electronic system. This is best accomplished by selecting the channel gates to fall on those portions of the energy spectrum which have minimal slopes.

The best compromise among stability, counting efficiency, maximal m and minimal n is obtained in the present instance by passing 0.3–1.2 mev pulses through channel 1 and 1.6–2.8 mev pulses through channel 2. The Na^{24} and K^{42} channel ratios are then $m=0.875$ and $n=0.015$. The counter sensitivity in channel 1 for samples diluted to 40 ml. in a 50 ml. Erlenmeyer flask placed directly above the scintillator is 1,000 c/s/μc. for Na^{24} and 200 c/s/μc. for K^{42}.

B. THE BIOLOGIC SYSTEM

The biologic system is similar in principle to previous methods and, with a few changes, follows the technic of Marcus, Romanoff and Pincus (1952). Male rats (Sprague-Dawley strain) were bilaterally adrenalectomized when they were between 80 and 90 gm. in weight. After adrenalectomy the rats were placed on regulation food pellets and saline solution and glucose ad libitum. They were used for assay when they were 100 gm. or more in weight, or not less than 3 days after operation. Thirty-two rats were removed for any one assay, which consisted of three groups of 8 animals each for three doses separated by log interval 0.4771 of the steroid in question, and one group of 8 animals as controls. At the time of the assay, the rats were removed from food and water, and the bladders were emptied by combined light ether anesthesia and suprapubic pressure. After the bladder had been emptied, each animal of the four groups was injected with 7.5 microcuries of potassium^{42} and 1.25 microcuries of sodium^{24} contained in 0.1 cc. solution. Both radioactive elements were present as carbonates. The amounts of the substances varied from 4–120 μg. Na_2Co_3, and from 0.4–15 mg. K_2Co_3. However, such variations in specific activity apparently had no effect on the assay. After injection of the animals, a tenth of the dose of the radioactive solution was placed into each of five 50 cc. Erlenmeyer flasks. The contents of the flasks were made up to 40 cc. with distilled water and served as samples of the dose for computation of the per cent of each isotope excreted. The test groups were then again injected with 0.1 cc. of 1:1 propylene glycol-water containing the adrenal steroid in question. A group of control rats were injected with 0.1 cc. of 1:1 propylene glycol-water. Immediately after injection, each rat was placed in an individual all-glass metabolic cage and maintained for 3 hours without food or fluid. At the end of this time, the bladders were emptied again, the urine was collected and the apparatus was washed down with distilled water so that the combined urine and wash made up to 40 cc. in Erlenmeyer flasks. All urine samples were now subjected to radioactive sodium and potassium determination. The per cent excretion of both Na^{24} and K^{42} of each animal was calculated by reference to the counts of the five samples of the dose. The ratio Na^{24}/K^{42} was calculated by dividing the per cent of dose Na^{24} excreted by a similar value of K^{42}. The ratio of the control animals was set at 100 per cent, and the per cent change of this ratio in the experimental groups was calculated by reference to the control ratio.

As an example of the foregoing, three doses of aldosterone[2] ranging from 0.025 to 0.25 microgram were assayed against three doses of desoxycorticosterone acetate ranging

[2] Kindly furnished by our colleagues, Drs. Mattox and Mason.

from 2.5 to 25 micrograms. The per cent change in ratio over suitable controls gave values which when plotted on semi-log paper, were fitted by eye to a straight line. From the two parallel lines thus obtained, the mean dose which reduced the ratio to 50 per cent of the control value ($\overline{\text{Ed}}$ 50) was interpolated. The mean effective dose for reduction of the ratio to one half of its control value for desoxycorticosterone acetate was 8.0 micrograms (96 animals) and 0.055 microgram for aldosterone (32 animals). Aldosterone was 145 times more active than the desoxycorticosterone acetate in this assay. Potency of this order of magnitude agrees fairly well with the value of 121 times the potency of desoxycorticosterone acetate (Speirs, Simpson and Tait, 1954).

SUMMARY

A method is described for the simultaneous determination of radioactive sodium and potassium in urine by gamma ray counting. The application of this physical method of determination of Na^{24} and K^{42} to the assay of adrenal steroids effective in mineral metabolism is illustrated. The advantages of gamma ray counting over beta ray counting are ease, convenience and rapidity of performing the determinations, and versatility for application to *in vivo* problems.

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BOOK REVIEW

THE BIOCHEMISTRY OF SEMEN. 1954. By T. Mann, M.D., Sc.D., Ph.D., F.R.S. Methuen's Monographs on Biochemical Subjects. Published by Methuen & Co. Ltd., London, and John Wiley & Sons, Inc., New York, pp. 240, 7 plates and 16 text figures. Price, $2.90.

This small volume is a masterly treatise of the subject of the biochemistry of semen by a distinguished scholar and investigator. Clearly, modestly and interestingly written, it gives not only a comprehensive survey of the historical development of the subject, but also an account of the most recent advances in the biochemistry of semen, in which Dr. Mann, himself, has played a leading part.

The book contains a preface, introduction, nine chapters, concluding remarks, references and an index. The contents can be usefully and briefly covered by citing the chapter headings: I. The two components of semen: spermatozoa and seminal plasma; II. Chemical and physical properties of whole ejaculated semen; III. The influence of extraneous factors, hormones, and environmental conditions; IV. Intracellular enzymes, metalloproteins, nucleoproteins, and other protein constituents of spermatozoa; V. Protein constituents and enzymes of the seminal plasma; VI. Lipids and their role in the metabolism of semen; VII. Fructose and fructolysis; VIII. Spermine, choline, ergothioneine, and certain other bases in semen; IX. Citric acid and inositol.

Dr. Mann was confronted with an opportunity to study semen some ten years ago, while he was working at the Molteno Institute, Cambridge, England. His statement regarding his decision to devote himself to the study of semen conveys very well the scope and atmosphere of the book. "I did not hesitate," he writes, "to give up my former pursuits in order to devote myself to experiments involving biological material which offers the investigator a chance, almost unique so far as mammalian tissues are concerned, of correlating chemical and metabolic findings with clearly defined and highly specific criteria of physiological activity, such as the motility and fertilizing capacity of the spermatozoa. Among other peculiarities which make semen such a fascinating and attractive object of study is that it represents an animal tissue with but a single type of cells, the spermatozoa, freely suspended in a fluid medium of some complexity, the seminal plasma, and not subject to cellular growth, division or multiplication; thus, making it feasible to express all one's metabolic measurements directly in terms of cell numbers, without recourse to cumbersome and often unreliable standards such as dry weight of tissue, nitrogen content, or indeed, any other of the commonly used metabolic indices. From the purely practical point of view, which matters greatly, the ability of spermatozoa to 'survive,' i.e. retain their remarkable properties under conditions of long-term storage *in vitro*, is of great importance. This in turn, gives one a chance of exploring at will and under well-defined conditions *in vitro*, the intricate chemical mechanism underlying the viability, and ultimately, the senescence, of living animal cells.

So far as the nutrition of spermatozoa is concerned, semen resembles more a suspension of microorganisms in a nutrient medium, than other animal tissues which rely for their nutrients on the blood supply. Nature has endowed the spermatozoa with the means of very efficient utilization of extraneous sources of energy, such as are accessible to the sperm cells either in their natural environment, the seminal plasma, or in the artificial storage media."

The most significant recent biochemical investigations of semen, in which Dr. Mann

has played a leading role, concern the presence of fructose and the mechanism of fructolysis in the male accessory reproductive glands. In 1945, he identified the seminal sugar by chemical methods as free D(-) fructose and, subsequently, he and his associates investigated the occurrence and site of its formation, and introduced its use as an indicator of male sex hormone activity ("the fructose test"). Furthermore, he investigated the relationship between blood glucose and seminal fructose, the enzymic mechanism of fructose formation, the anaerobic and aerobic utilization of carbohydrate by spermatozoa, and the intermediary reactions in sperm fructolysis and the role of phosphorus containing co-enzymes. More recently he and his coworkers have begun to investigate two other constituents of semen, namely, citric acid and inositol.

The book contains interesting historical side-lights too numerous to mention. The history of spermine is one of the more fascinating of these accounts.

The bibliography includes nearly one thousand references and the index is comprehensive. The volume could usefully serve as a model for monographs relating biochemistry to biological material.

George B. Wislocki

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

Those wishing to present papers, (time limited to ten minutes) should send four copies of the title and abstract to the Vice-President, Dr. E. B. Astwood, 30 Bennet Street, Boston 11, Massachusetts, not later than *February 1, 1955*. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. Neither footnotes nor acknowledgments to sponsors can be published. References, if used, should be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading should be arranged as follows:
   - Line 1. Title, not to exceed fifteen words.
   - Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   - Line 3. Institute of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the head-

ing. The original copy should be on bond paper. There should be three copies.

4. Abstracts should be letter-perfect, as there will be no opportunity for proof reading by authors.
5. Abstracts not conforming to the foregoing regulations will be returned to the authors.

THE SEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE INDIANA UNIVERSITY SCHOOL OF MEDICINE AT THE CONTINUATION STUDY CENTER OF THE INDIANA UNIVERSITY MEDICAL CENTER, INDIANAPOLIS, INDIANA— SEPTEMBER 26–OCTOBER 1, 1955

The faculty will consist of twenty-one eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies with emphasis on the clinical aspects, with demonstrations of laboratory tests, presentations of cases, and question and answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program, write Post Graduate Office, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis 7, Indiana. Registration is limited to 100; tuition fee is $100.00. Arrangements for housing accommodations will be made through the Indiana Medical Center.